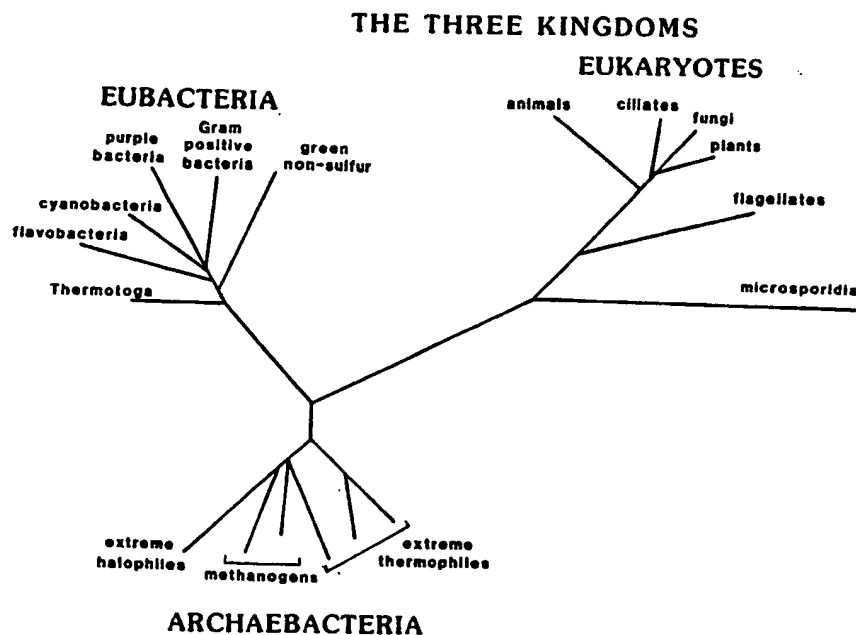




## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>5</sup> : <b>C12Q 1/68</b>	<b>A1</b>	(11) International Publication Number: <b>WO 90/15157</b> (43) International Publication Date: 13 December 1990 (13.12.90)
(21) International Application Number: PCT/US90/03004 (22) International Filing Date: 31 May 1990 (31.05.90)  (30) Priority data: 359,158                      31 May 1989 (31.05.89)                      US  (71) Applicant: GENE-TRAK SYSTEMS [US/US]; 31 New York Avenue, Framingham, MA 01701 (US).  (72) Inventors: LANE, David, J. ; 9 Oriole Drive, Milford, MA 01757 (US). SHAH, Jyotsna ; 13 Bates Drive, Nashua, NH 03060 (US). BUHARIN, Amelia ; 7 Pond Street, Framingham, MA 01701 (US). WEISBURG, William, G. ; 3 Jillson Circle, Milford, MA 01757 (US).	(74) Agent: JANIUK, Anthony, J.; Gene-Trak Systems, 31 New York Avenue, Framingham, MA 01701 (US).  (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).  <b>Published</b> <i>With international search report.          Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

(54) Title: UNIVERSAL EUBACTERIA NUCLEIC ACID PROBES AND METHODS



## (57) Abstract

Nucleic acid probes capable of hybridizing to rRNA of eubacteria and not to rRNA of non-eubacteria are described along with methods utilizing such probes for the detection of eubacteria in clinical and other samples. Preferred embodiments include probes capable of distinguishing between gram-positive and gram-negative bacteria.

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## UNIVERSAL EUBACTERIA NUCLEIC ACID PROBES AND METHODS

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## Field of the Invention

10 This invention relates to detection of bacteria in clinical and other samples. Methods for the detection of bacteria in ordinarily aseptic bodily tissues or fluids such as blood, urine, and cerebrospinal fluid - where the presence of any bacterium may be life threatening are of particular importance. The present invention provides nucleic acid probes and compositions along with methods for their use for the  
15 specific detection of any bacterium in such samples.

## Background of the Invention

20 The term "eubacteria" as used herein, refers to the group of prokaryotic organisms (bacteria) as described in, for example, Bergey's Manual of Systematic Bacteriology (N.R. Krieg and J.G. Holt, ed., 1984, Williams & Wilkins, Baltimore). As a group, the eubacteria comprise all of the bacteria which are known to cause disease in humans or  
25 animals and are of most concern with respect to detection.

The only other described group of bacteria, the archaeobacteria, are biologically and genetically distinct from the eubacteria (C.R. Woese, Scientific American, 1981, Volume 244, pages 98-102). Archaeobacteria as  
30 a group occupy a variety of "extreme" environments such as hot springs, strongly oxygen-depleted muds, salt brines, etc., which generally do not support the growth of eubacteria. There are no known archaeobacterial pathogens and, consequently, their detection is of little clinical significance.

Eukaryotic organisms comprise the third fundamental genetic lineage which, together with the eubacteria and archaebacteria, include all known life forms (Figure 1). Eukaryotes include humans, animals, plants and a host of organizationally less complex, free-living and parasitic "protists," including: protozoans, fungi, ciliates, etc. In a clinical context, it is particularly important that eubacteria be distinguished from eukaryotic, e.g. fungal and protozoan, infections which may present the same symptoms but require a significantly different regime of antimicrobial or chemo-therapy. These genetic distinctions thus are clinically significant from the point of view of diagnosis and antimicrobial chemotherapy.

It is an aspect of the present invention to provide nucleic acid probes which discriminate between eubacterial, human (including human mitochondrial) and fungal rRNA molecules.

It is another aspect of the present invention to provide probes and probe sets which provide a basis for discriminating between Gram positive and Gram negative eubacteria.

Methods for detecting, identifying and enumerating bacteria in normally sterile body fluids vary with the type of sample and the suspected pathogen. No currently available method is optimal for the detection of all pathogens. Often a combination of methods must be used to increase the likelihood that the pathogen will be detected. All commonly used methods for detection of, for example, bacteremia or bacterial septicemia rely on the in vitro cultivation of microbes from clinical samples. Generally, a blood sample is drawn from a patient and incubated in a rich artificial culture medium and monitored for 1 to 14 days. During this time, the medium is examined or blindly subcultured (plated), or assayed chemically or isotopically for evidence of bacterial growth or fermentative processes. Clinicians generally draw two or three samples of 10 milliliters of blood which may yield as

few as one to ten colony forming units of bacteria for a positive diagnosis. Following the isolation of individual colonies of bacteria on diagnostic solid media and/or by Gram-staining, presumptive identification of the bacteria (or fungus) is made.

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All cultivation methods suffer a number of serious shortcomings, including the following:

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- High material costs;

- Labor intensive;

- Technologists extensively handle dangerous bodily fluids;

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- False positives due to handling;

- False negatives due to low viable cell numbers;

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- False negatives due to fastidious media requirements of many potential pathogens; and

- Relatively long time to positive diagnosis and identification.

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Because of the relatively long time required by current methods to achieve a diagnosis and because of the potentially life threatening nature of such infections, antimicrobial therapy often is begun empirically before the results of such tests can be known.

30

Therefore, it is another aspect of the present invention to provide nucleic acid probes which are broadly specific for all eubacteria and which preferably do not react with other eukaryotic pathogens, especially fungi, which may be present in sampled materials.

It is yet another aspect of the present invention to provide probes which may be used in a variety of assay systems which avoid many of the disadvantages associated with traditional, multi-day culturing techniques.

5

It is still another aspect of the present invention to provide probes that are capable of hybridizing to the ribosomal ribonucleic acid (rRNA) of the targeted eubacterial organisms under normal assay conditions.

10

While Kohne et al. (Biophysical Journal 8:1104-1118, 1968) discuss one method for preparing probes to rRNA sequences, they do not provide the teaching necessary to make broad-specificity eubacterial probes.

15

Pace and Campbell (Journal of Bacteriology 107:543-547, 1971) discuss the homology of ribosomal ribonucleic acids from diverse bacterial species and a hybridization method for quantitating such homology levels. They do not identify particular nucleic acid sequences shared by bacteria, but absent in eukaryotes. Woese (Microbiological Reviews 51:221-271, 1987) describes the breadth of the eubacteria, in terms of rRNA sequence, but does not indicate sequences of interest for complete bacterial inclusivity. These references, however, fail to relieve the deficiency of Kohne's teaching with respect to eubacterial probes and, in particular, do not provide eubacterial specific probes useful in assays for detecting eubacteria in clinical or other samples.

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25

Giovannoni et al. (Journal of Bacteriology 170:720-726, 1988) describe a number of probes which are claimed to be useful for the identification of broad groups of eubacteria, archaeobacteria and eukaryotes. However, Giovannoni et al. do not disclose the probes of the present invention. Nor do they provide the teaching necessary to design such probes.

30

Hogan et al. (European patent publication WO 88/03957) describe a number of probes which are claimed to hybridize to a broad representation of eubacteria. However, Hogan et al. do not teach the probes of the present invention and also fail to relieve the deficiency of Kohne's teaching with respect to these probes.

Ribosomes are of profound importance to all organisms because they serve as the only means of translating genetic information into cellular proteins. A clear manifestation of this importance is the observation that all cells have ribosomes. Actively growing bacteria may have 20,000 or more ribosomes per cell. This makes ribosomes one of the most abundant macromolecular entities in a cell, and an attractive diagnostic assay target.

Ribosomes contain three distinct RNA molecules which in Escherichia coli are referred to as 5S, 16S and 23S rRNAs. These names historically are related to the size of the RNA molecules, as determined by their sedimentation rate. In actuality, however, ribosomal RNA molecules vary in size between organisms. Nonetheless, 5S, 16S, and 23S rRNA are commonly used as generic names for the homologous RNA molecules in any bacteria, and this convention will be continued herein. Discussion will be confined to 16S and 23S rRNAs.

As used herein, probe(s) refer to synthetic or biologically produced nucleic acids (DNA or RNA) which, by design or selection, contain specific nucleotide sequences that allow them to hybridize under defined predetermined stringencies, specifically (i.e., preferentially, see below - Hybridization) to target nucleic acid sequences. In addition to their hybridization properties, probes also may contain certain constituents that pertain to their proper or optimal functioning under particular assay conditions. For example, probes may be modified to improve their resistance to nuclease degradation (e.g. by end capping), to carry detection ligands (e.g. fluorescein, 32-Phosphorous, biotin, etc.), or to facilitate their capture onto a solid

The detection of bacteria by nucleic acid hybridization constitutes enhanced performance capability compared to the available culture-dependent tests for several reasons including:

- 5           a) increased sensitivity; i.e., the ability to detect said bacteria in a given sample more frequently;
- b) potentially significant reductions in assay cost due to the use of inexpensive reagents and reduced labor;
- 10           c) accurate detection of even nutritionally fastidious strains of bacteria;
- d) faster results because such tests do not require the isolation of the target bacterium from the sample prior to testing;
- 15           e) the ability to screen, in a batch mode, a large number of samples, and only culture those identified as "hybridization positive";
- 20           f) potential detection of phagocytized organisms eliminating the need for multiple, punctuated blood samples in order to sample the cyclical "window" of viable organisms (which probably depends on host immunological cycles);
- 25           g) some reduction of technologist handling of potentially infectious body fluids;
- h) the ability to detect very low numbers of targets by amplifying either the bacterial signal or target using in vitro nucleic acid amplification.
- 30

It has been discovered that other advantages incurred by directing the probes of the present invention against rRNA include the fact that the rRNAs detected constitute a significant component of cellular mass.

5 Although estimates of cellular ribosome content vary, actively growing Escherichia coli, for example, may contain upwards of 50,000 ribosomes per cell, and therefore 50,000 copies of each of the rRNAs (present in a 1:1:1 stiochiometry in ribosomes). The abundance of ribosomes in other bacteria particularly under other, less favorable, metabolic conditions may be considerably lower. However, under any  
10 circumstances, rRNAs are among the most abundant cellular nucleic acids present in all cell types. In contrast, other potential cellular target molecules such as genes or RNA transcripts thereof, are less ideal since they are present in much lower abundance.

15 A further unexpected advantage is that the rRNAs (and the genes specifying them) appear not to be subject to lateral transfer between contemporary organisms. Thus, the rRNA primary structure provides an organism-specific molecular target, rather than a gene-specific target as would likely be the case, for example of a plasmid-borne gene or  
20 product thereof which may be subject to lateral transmission between contemporary organisms.

Additionally, the present invention provides probes to eubacterial rRNA target sequences which are sufficiently similar in most or all  
25 eubacteria tested that they can hybridize to the target region in such eubacteria. Advantageously, these same rRNA target sequences are sufficiently different in most non-eubacterial rRNAs that, under conditions where the probes hybridize to eubacterial rRNAs they do not hybridize to most non-eubacterial rRNAs. These probe characteristics  
30 are defined as inclusivity and exclusivity, respectively.

The discovery that probes could be generated with the extraordinary inclusivity and exclusivity characteristics of those of the present invention with respect to eubacteria was unpredictable and unexpected.

10

### Brief Description of the Figures

- 5 Further understanding of the principles and aspects of the present invention may be made by reference to the tables wherein:

10 Figure 1 - Shows an evolutionary "tree" of the major genetic "kingdoms" of life (Woese, 1987, Microbiological Reviews 51:221- 271). The branching patterns represent the mutational distances between the 16S rRNA sequences of the represented organism. Such comparisons readily distinguish the eubacteria from the archaebacteria and eukaryotes.

15 Figure 2 - Shows a more detailed evolutionary tree of the eubacterial kingdom (ibid.). So far about 10 major divisions/phyla have been defined based on 16S rRNA sequence comparisons. Certain discriminations among eubacterial divisions can be important in a clinical context and certain of the probes of the present invention do exhibit preferential hybridization to one or more of the eubacterial divisions. Therefore, the test organisms listed in Tables 3, 4 and 5 are grouped according to the divisions shown in Figure 2 so that significant patterns of hybridization may be most easily discerned.

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### Brief Description of the Tables

Table 1 - Shows alignment of the nucleotide sequences of the preferred 16S rRNA-targeted probes of the present invention with their target nucleotide sequences in E. coli 16S rRNA. Very extensive sequence comparison to some 350 aligned 16S and 18S rRNA sequences were performed during the development of the probes of the present invention. It simply is not practical to show this analysis in detail. However, a consensus sequence (CONS-90%) of highly conserved 16S rRNA nucleotide positions is provided as a summary of the patterns of

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nucleotide sequence variation discovered among representative eubacteria. A nucleotide on the CONS-90% line indicates that that nucleotide is found at the homologous position in 90% or greater of the eubacterial sequences inspected. Note that the probe target regions all correspond to clusters of high sequence conservation among the eubacterial 16S and 23S rRNA molecules.

Since the E. coli 16S and 23S rRNA sequences were among the first full rRNA sequences obtained, the assigned position numbers have become a convenient and commonly accepted standard for explicitly identifying the homologous regions in other rRNA sequences under consideration. In Table 1, the E. coli RNA (target) sequence is written 5' to 3'. Probe sequences are DNA and written 3' to 5', except for probes 1638, 1642 and 1643 which are designed to hybridize to the rRNA-complementary sequence rather than the rRNA itself. These latter probes have the same "sense" (i. e. polarity) as the rRNA and are written 5' to 3'.

Table 2 - Shows alignment of the nucleotide sequences of the preferred 23S rRNA-targeted probes of the present invention with their target nucleotide sequences in E. coli 23S rRNA. As in Table 1 the E. coli sequence numbering is used as a standard in order to identify the homologous probe target sequences in all 23S rRNAs. CONS-90% has the same meaning as in TABLE 1. For the 23S rRNA analyses only about 30 sequences were available. However, these represent most of the major eubacterial divisions shown in Figure 2. In the probe 1730 sequence, "R" = a 1:1 mixture of A and G at that position.

Table 3 - Exemplifies the inclusivity and exclusivity behavior of a number of the preferred 16S rRNA-targeted probes toward a representative sampling of eubacterial and non-eubacterial rRNAs in a dot blot hybridization assay.

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Table 4 - Exemplifies the inclusivity and exclusivity behavior of a number of the preferred 23S rRNA-targeted probes toward a representative sampling of eubacterial and non-eubacterial rRNAs in a dot blot hybridization assay.

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Table 5 - Exemplifies the inclusivity and exclusivity behavior of a number of additional preferred 16S and 23S rRNA-targeted probes toward a representative sampling of eubacterial and non-eubacterial rRNAs in a dot blot hybridization assay. These probes exhibit useful patterns of hybridization to specific subgroups of eubacteria - notably Gram positive and Gram negative bacteria.

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#### Detailed Description of the Invention and Best Mode

##### Probe Development Strategy:

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The first step taken in the development of the probes of the present invention involved identification of regions of 16S and 23S rRNA which potentially could serve as target sites for eubacteria specific nucleic acid probes. This entailed finding sites which are:

25

1) highly conserved (few nucleotide changes, deletions, or insertions) among eubacterial rRNA sequences, and

2) substantially different in non-eubacterial rRNA sequences.

30

For this analysis, precise alignments of available 16S and 23S rRNA sequences were developed. A number of 16S and 23S rRNA sequences were determined as part of this effort. Such nucleotide sequences were determined by standard laboratory protocols either by cloning (Maniatis et al., 1982, Molecular Cloning; A Laboratory Manual, Cold Spring Harbor Laboratory, New York, pp 545) and sequencing (Maxam and Gilbert,

1977, Proceedings of the National Academy of Science, USA 74:560-564;  
Sanger et al., 1977, Proceedings of the National Academy of Science,  
USA 74:5463-5467) the genes which specify the rRNAs, and/or by direct  
sequencing of the rRNAs themselves using reverse transcriptase (Lane et  
5 al., 1985, Proceedings of the National Academy of Science, USA 82:6955-  
6959; Lane, manuscript in preparation).

A computer algorithm, operating on the aligned set of 16S and 23S rRNA  
sequences, was used to identify regions of greatest similarity among  
10 eubacteria. Nucleic acid probes to such regions will hybridize most  
widely among diverse eubacteria.

Such regions of homology among eubacteria next were assessed for  
differences with non-eubacterial rRNA sequences. In particular,  
15 sequence differences between eubacterial and human, fungal, and  
mitochondrial sequences were sought.

Forty one probes were designed based on these analyses; 22 targeting  
23S rRNA and 19 targeting 16S rRNA.  
20

The hybridization behavior of these probes toward extensive panels of  
eubacteria was determined by hybridization analysis in a dot blot  
format.

#### 25 Physical Description of the Probes:

The foregoing probe selection strategy yielded a number of probes  
useful for identifying eubacteria in samples and include the following  
preferred oligonucleotide probes:

30

16S rRNA-targeted probes:

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- Probe 1638: 5'-AGAGTTTGATCCTGGCTCAG-3'
- 5 Probe 1642: 5'-AGAGTTTGATCATGGCTCAG-3'
- Probe 1643: 5'-AGAGTTTGATCCTGGCTTAG-3'
- Probe 1738: 5'-CTGAGCCAGGATCAAACCTCT-3'
- 10 Probe 1744: 5'-CAGCGTTGCTCCTGAGCCAGGATCAAACCT-3'
- Probe 1659: 5'-CTGCTGCCTCCCGTAGGAGT-3'
- Probe 1660: 5'-CTGCTGCCTCCCGTAGGAGTTTGGGCCGTGTCTCAGTTCCAGTGT-3'
- 15 Probe 1661: 5'-TATTACCGCGGCTGCTGGCACGGAGTTAGCCG-3'
- Probe 1739: 5'-GCGTGGACTACCGGGGTATCTAATCCTGTTTGCTCCCCACGCTTTTCG-3'
- 20 Probe 1740: 5'-GGGTTGCGCTCGTTGCGGGACTTAACCCGACATCTCACGGCAGGAGCT  
GACGACAGCCATGCAT-3'
- Probe 1741: 5'-CTCACGGCAGGAGCTGACGACAGCCATGCAT-3'
- 25 Probe 1742: 5'-GGGTTGCGCTCGTTGCGGGACTTAACCCGACAT-3'
- Probe 1745: 5'-AGCTGACGACAACCATGCACCACCTGT-3'
- Probe 1746: 5'-TCATAAGGGGCATGATGATTTGACGTCAT-3'
- 30 Probe 1743: 5'-GTACAAGGCCCGGAACGTATTACCG-3'
- Probe 1637: 5'-AAGGAGGTGATCCAGCC-3'

15

Probe 1639: 5'-ACGGTTACCTTGTTACGACTT-3'

Probe 1640: 5'-ACGGCTACCTTGTTACGACTT-3'

5 Probe 1641: 5'-ACGGATACCTTGTTACGACTT-3'

23S rRNA-targeted probes:

10 Probe 1730: 5'-CTTTTCCTTTCCCTCRGGTACTGGTTCRCTATCGGTC-3'

Probe 1731: 5'-CTTTTCGCCTTTCCCTCGGGTACTGGTTCGCTATCGGTC-3'

Probe 1658: 5'-TCTTTAAAGGTGGCTGCTTCTAAGCCAACATCCTGGTTG-3'

15 Probe 1656: 5'-CTACCTGTGTCGGTTTGGGTACGGGC-3'

Probe 1657: 5'-GGTATTCTCTACCTGACCACCTGTGTCGGTTTGGGTACG-3'

20 Probe 1653: 5'-CCTTCTCCCGAAGTTACGGGGGCATTTGCCTAGTTCCTT-3'

Probe 1654: 5'-CCTTCTCCCGAAGTTACGGGGTCATTTGCCGAGTTCCTT-3'

Probe 1655: 5'-CCTTCTCCCGAAGTTACGGCACCATTTGCCGAGTTCCTT-3'

25 Probe 1651: 5'-CTCCTCTTAACCTTCCAGCACCGGGCAGGC-3'

Probe 1652: 5'-TTCGATCAGGGGCTTCGCTTGGCGTGACCCCATCAATTAA-3'

30 Probe 1512: 5'-TTAGGACCGTTATAGTTACGGCCGCGTTTACTGGGGCTT-3'

Probe 1256: 5'-GGTCGGAACCTTACCCGACAAGGAATTCGCTACCTTAG-3'

Probe 1398: 5'-GGTCGGTATTTAACCGACAAGGAATTCGCTACCTTAG-3'

16

Probe 1511: 5'-CGTGCGGGTCGGAACTTACCCGACAAGGAATTCGCTACC3'

Probe 1595: 5'-CGATATGAACTCTTGGGCGGTATCAGCCTGTTATCCCCGG-3'

5 Probe 1600: 5'-CAGCCCCAGGATGAGATGAGCCGACATCGAGGTGCCAAAC-3'

Probe 1601: 5'-CAGCCCCAGGATGTGATGAGCCGACATCGAGGTGCCAAAC-3'

Probe 1602: 5'-CAGCCCCAGGATGCGATGAGCCGACATCGAGGTGCCAAAC-3'

10

Probe 1598: 5'-CGTACCGCTTTAAATGGCGAACAGCCATACCCTTGGGACC-3'

Probe 1599: 5'-CGTGCCGCTTTAAATGGGCGAACAGCCCAACCCTTGGGACC-3'

15 Probe 1596: 5'-GATAGGGACCGAACTGTCTCAGACGTTTGAACCCAGCT-3'

Probe 1597: 5'-GATAGGGACCGAACTGTCTCAGACGTTCTGAACCCAGCT-3'

20 The specific behaviors of the aforementioned probes are dependent to a significant extent on the assay format in which they are employed. Conversely, the assay format will dictate certain of the optimal design features of particular probes. The "essence" of the probes of the invention is not to be construed as restricted to the specific string of nucleotides in the named probes. For example, the length of these  
25 particular oligonucleotides was optimized for use in the dot blot assay (and certain other anticipated assays) described below. It is well known to those skilled in the art that optimal probe length will be a function of the stringency of the hybridization conditions chosen and hence the length of the instant probes may be altered in accordance  
30 therewith. Also, in considering sets comprised of more than one probe, it is desirable that all probes behave in a compatible manner in any particular format in which they are employed. Thus, the exact length

of a particular probe will to a certain extent, reflect its specific intended use. Again, given the probes of the instant invention, these are familiar considerations to one of ordinary skill in the art.

- 5     The "essence" of the probes described herein resides in the discovery and utilization of the specific sequences described above and given in Table 1 and Table 2.

Hybridization Analysis of Probe Behavior:

- 10     The sequence comparisons which led to the discovery of the disclosed target sequences suggested that many of the probes should hybridize to a significant number of eubacteria. For the 16S rRNA analyses, some 350 sequences were considered in designing the probes; for the 23S rRNA
- 15     analyses only about 30 eubacterial sequences were available. Since it is impossible to test every eubacterial strain, greater sequence variation might exist in other eubacterial strains not inspected by sequence analysis which might reduce or eliminate hybridization by the prospective probes to such untested eubacteria. As can be seen in
- 20     Tables 3, 4 and 5, some probes of extremely broad inclusivity nevertheless fail to hybridize to certain bacteria. Therefore, carefully documenting the hybridization behavior to a large and representative number of eubacteria is an important element in documenting that such probes are capable of detecting all eubacteria
- 25     or, failing that, for documenting which eubacteria are not detected. Such "failures" may not be clinically significant or alternatively, may be compensated for by appropriate inclusion of other probes of the instant inventions.
- 30     Equally as important as the inclusivity behavior of the probes, is their exclusivity behavior, i.e., their reactivity toward non-eubacteria. As mentioned, demonstrating a lack of hybridization to human and fungal rRNAs is of paramount importance in the types of clinical applications envisioned for such probes. Therefore, the

behavior of the probes toward representative eubacterial, human and fungal rRNAs was determined by hybridization analysis using a dot blot procedure.

5     **Example 1: Dot-blot analysis of probe hybridization behavior.**

Dot-blot analysis, in accordance with well known procedures, involves immobilizing a nucleic acid or a population of nucleic acids on a filter such as nitrocellulose, nylon, or other derivatized membranes which readily can be obtained commercially, specifically for this purpose. Either DNA or RNA can be easily immobilized on such a filter and subsequently can be probed or tested for hybridization under any of a variety of conditions (i.e., stringencies) with nucleotide sequences or probes of interest. Under stringent conditions, probes whose nucleotide sequences have greater complementarity to the target sequence will exhibit a higher level of hybridization than probes containing less complementarity. For most of the oligonucleotide probes described herein, hybridization to rRNA targets at 60°C for 14-16 hours (in a hybridization solution containing 0.9 M NaCl, 0.12 M Tris-HCl, pH 7.8, 6 mM EDTA, 0.1 M KPO<sub>4</sub>, 0.1% SDS, 0.1% pyrophosphate, 0.002% ficoll, 0.02% BSA, and 0.002% polyvinylpyrrolidone), followed by standard post-hybridization washes to remove unbound and non-specifically hybridized probe (at 60°C in 0.03 M NaCl, 0.004 M Tris-HCl, pH 7.8, 0.2 mM EDTA, and 0.1% SDS), would be sufficiently stringent to produce the levels of specificity demonstrated in Tables 3, 4 and 5. The exceptions to these conditions are probe 1738 (which was hybridized at 37°C), and probe 1746 (which was hybridized at 37°C and washed at 50°C).

30     Techniques also are available in which DNA or RNA present in crude (unpurified) cell lysates can be immobilized without first having to purify the nucleic acid in question (e.g. Maniatis, T., Fritsch, E. F. and Sambrook, J., 1982, Molecular Cloning: A Laboratory Manual).

- The dot-blot hybridization data shown in Tables 3, 4 and 5 were generated by hybridization of the indicated probes to purified RNA preparations from the indicated eubacterial, fungal and human specimens. Bacterial and fungal RNAs were purified from pure cultures of the indicated organisms. Mouse RNA was purified from L cells (a tissue culture cell line). Wheat germ RNA was purified from a commercial preparation of that cereal product. Human blood and stool RNAs were purified from appropriate specimens obtained from normal, healthy individuals.
- Purified RNA was used, rather than cell lysates for a number of simple technical reasons. The most important of these relate to proper interpretation of the relative signal arising from the hybridization of any particular probe to individual organisms. RNA content per cell is known to vary widely among different bacteria and varies even more between bacteria and eukaryotic cells. In addition, the specific metabolic status of cells at the time of harvest can have a profound influence on the amount and integrity of the RNA recovered. Some bacteria, for example, begin to degrade their RNA very rapidly upon reaching the stationary growth phase. The organisms represented in Tables 3, 4 and 5 comprise an extremely diverse collection in every respect. Represented are Gram positive and Gram negative bacteria, photosynthetic and chemosynthetic, heterotrophic and lithotrophic, and anaerobic and aerobic metabolisms. By using known, equivalent amounts of purified RNA in the individual "dots," relative levels of hybridization of each probe to each organism can be meaningfully compared without regard to the idiosyncracies of nucleic acid preparation from individual types of bacteria represented.
- RNA was prepared by a variation on standard published methods which has been developed in our laboratory (W. Weisburg, unpublished). The method rapidly yields bulk high molecular weight RNA in a highly purified but relatively unfractionated form. Little or no DNA, or low molecular weight RNA species are found in RNA prepared in this fashion.

A large proportion of the RNA is 16S and 23S rRNA (18S and 28S rRNA in eukaryotes) as is true of the RNA in the intact cells. The method is rapid and convenient, but otherwise is not relevant to interpretation of the dot-blot results presented in Tables 3, 4 and 5. Most other  
5 currently accepted methods available in the literature which yield RNA of reasonable intactness will yield equivalent hybridization results.

For the hybridization experiments reported in Tables 3, 4 and 5, probes were end-labeled with radioactive 32-phosphorous, using standard  
10 procedures. Following hybridization and washing as described above, the hybridization filters were exposed to X-ray film and the intensity of the signal evaluated with respect to that of control RNA spots containing known amount of target RNA of known sequence.

15 A scale of hybridization intensity ranging from ++++ (hybridization signal equivalent to that of control spots) to + (barely detectable even after long exposure of the x-ray film) has been used to compare hybridization signals between organisms and probes. +++ signal indicates a very strong signal only slightly less intense than control  
20 spots. ++ indicates a clearly discernible hybridization signal, but one that is noticeably weaker than the control spots. Note that while more "quantitative" ways to record hybridization signal are available, they are much more cumbersome to employ and, in our experience, not really any more useful for probe evaluation than the method employed in  
25 Tables 3, 4 and 5. In fact, because of certain uncontrollable variables in spotting exactly equivalent amounts of target RNA (of equivalent intactness) from such disparate organisms, numerically more precise counting methods are only deceptively more quantitative. In our experience, an organism generating a ++ or greater signal to a  
30 particular probe is easily distinguished from one generating a "-" signal. This is true of a variety of assay formats that have been tested.

As is evident in Tables 3 and 4, 23S rRNA-targeted probes 1600, 1602, 1596, 1256 and 1512 and 16S rRNA-targeted probes, 1738, 1660, 1639, 1739, 1740, 1741 and 1743 hybridize most extensively among the eubacteria and are thus the most preferred. Other probes hybridize in a variety of patterns to subgroups of eubacteria and would be preferred for the detection of those subgroups or as components of more broadly inclusive probe sets. For example, probes 1599, 1656, 1744, 1745 and 1746 hybridize preferentially to Gram positive bacteria. Probes 1657, 1598 and 1595 hybridize preferentially to gram-negative bacteria, particularly to members of the so-called "purple bacterial" division (Figure 2 and Table 5).

Other probes exhibit other useful patterns of hybridization as is evident upon inspection of the data in Tables 3, 4 and 5. These probes can be combined in a variety of ways to create probe sets which exhibit the combined hybridization properties of the component probes. An example of one such hybridization format is given below (Example 2).

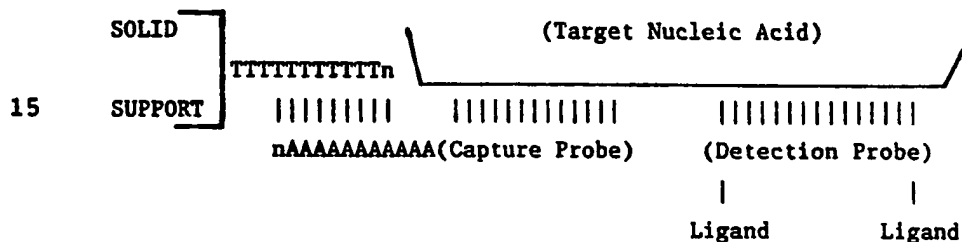
Alternatively, the probes could be used in a variety of subtractive hybridization schemes in which specific rRNA molecules are removed from the pool present in a mixed population of organisms prior to or simultaneous with the target organism-specific probes (e.g. Collins, European Patent Application 87309308.2).

#### Example 2: Dual Probe, Sandwich Hybridization Assay

The probes of the present invention or derivatives thereof can be advantageously employed in a variety of other hybridization formats. One such format is a dual probe, sandwich-type hybridization assay such as that described, for example, in USSN 277,579; USSN 169,646, or USSN 233,683. In such a dual probe application, one probe (for example, probe 1602 or a derivative) would be ideally modified at its 3' terminus to contain a tract of about 20 - 200 deoxyadenosine (dA) residues. This would be used to "capture" the target rRNA (following

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liquid hybridization) from the test sample onto a solid support (e.g., beads, plastic surface, filter, etc.) which had been suitably derivatized with poly-deoxythymidine (dT) for this purpose. A second probe (for example, probe 1596 or derivative) would then be advantageously used as the detection probe and would be suitably derivatized with some detectable ligand (e.g. 32-P, fluorescein, biotin, etc.). Detection of the presence of the target nucleic acid in a test sample then would be indicated by capture of the detection ligand onto the solid surface through the series of hybridization interactions:



This could occur only if the target nucleic acid is present in the test sample. In principle, the above scheme could be employed with multiple capture and detection probes (probe sets) for the purpose of, for example, improving inclusivity or enhancing sensitivity of the assay.

Example 3: PCR Amplification of 16S rRNAs.

The polymerase chain reaction (PCR) is a well known method for amplifying target nucleic acid by "copying" the nucleic acid sequences located between two target sequences (US 4,683,202). The PCR process could be useful in an assay for the diagnosis of, for example, a non-viral pathogen by amplifying the genes encoding the pathogen's rRNA or rRNA genes and subsequently detecting that product. Implementation of this diagnostic strategy requires the invention of primers capable of

amplifying the rRNA of the targeted organism(s). A second important application of such primers is in cloning amplified rRNA genes, and a third application is the direct sequencing of amplified rRNA genes.

5 Probes 1638, 1642, 1643, 1637, 1639, 1640 and 1641 may be ideally used as primers for enzymatically copying and/or amplifying eubacterial 16S rRNAs or the genes encoding them. Details of the PCR procedure vary slightly depending on whether the target nucleic acid is single or double stranded, and whether it is DNA or RNA. However, the principle  
10 is the same in either case. Briefly, the steps are as follows:

- 1) Double-stranded DNA is denatured,
- 15 2) Oligonucleotide primers complimentary to each of the sister DNA strands are annealed, and
- 20 3) deoxynucleotide triphosphate precursors are incorporated into newly synthesized sister DNA strands by extension of the primers from their 3' termini using DNA polymerase and/or reverse transcriptase.

Thus, a pair of oligonucleotide primers are required for the PCR reaction, one complementary to each strand within the target gene. They are positioned such that the newly synthesized product of one  
25 primer is also a target/template for the other primer. Thus the target nucleotide sequence located between the two primer annealing sites may be amplified many fold by repeating the steps listed above 20 to 30 times.

30 Probes 1638, 1642, 1643, 1637, 1639, 1640 and 1641 are suitable for use as primers for enzymatically copying and/or amplifying eubacterial 16S rRNAs or the genes encoding them. That is, as a set, they will anneal very broadly among eubacterial rRNAs and rRNA genes and so will amplify any eubacterial rRNA sequences present in a sample.

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Probes 1637, 1639, 1640 and 1641 hybridize to the 16S rRNA (or rRNA-like strand of the ribosomal RNA gene) near its 3' end (Table 1). The template strand is read in the 3' to 5' direction producing an rRNA-complementary strand with the primer itself incorporated at its 5' terminus.

Probes 1638, 1642, and 1643 hybridize near the 5' end of the rRNA-complementary strand of the rRNA gene or to such a complement produced as described immediately above.

Individually, the above-described 16S rRNA amplification primers have approximately the following specificities:

5' primers:

Probe 1638: most eubacteria

Probe 1642: enterics and relatives

Probe 1643: Borrelia spirochetes

3' primers:

Probe 1637: most eubacteria

Probe 1639: enterics, Deinococcus, Campylobacter

Probe 1640: most eubacteria

Probe 1641: fusobacteria, some Bacillus species

In test samples where the target bacterium is known, specific primers can be used. Where the target organism is not specifically known (for example, any eubacterium) all of the above mentioned primers can be used as a set.

5

The above described primers have been designed to amplify nearly the entire 16S rRNA sequence. Any of the other probes of the present invention or derivatives thereof can be used to amplify sub-segments of the 16S and 23S rRNAs or genes in a fashion similar to that just described.

10

Any such primers can be modified in a great number of ways to, for example, incorporate RNA polymerase promoters, cloning sites, etc. into the amplified transcripts.

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While the description of the invention has been made with reference to detecting rRNA, it will be readily understood that the probes described herein and probes complementary to those described herein also will be useful for the detection of the genes (DNA) which specify the rRNA and, accordingly, such probes are to be deemed equivalents to the described probes and encompassed within the spirit and scope to the present invention and the appended claims.

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TABLE 1: 16S rRNA-TARGETED PROBES AND TARGET SEQUENCES

E. coli#s	8	38
CONS-90%	ACAGUUUGAUC UGGUCAG	GAACCCUGGCG
E. coli	5'-AAAUUGAAGAGUUUGAUCAGUCUCAGAUUGAACCCUGGCG	
Probe 1738	3'-TCTCAAACTAGCAGCGAGTC-5'	
Probe 1744	3'-TCAAACTAGGACCGAGTCCTGCTTGGAC-5'	
Probe 1638	5'-AGAGTTTGATCCTGGCTCAG-3'	
Probe 1642	5'-AGAGTTTGATCATGGCTCAG-3'	
Probe 1643	5'-AGAGTTTGATCCTGGCTTAG-3'	
E. coli#s	313	357
CONS-90%	G CACA UGG ACUGAGACACGG CCA ACUCCUAGGGAGGCAGCAGU	
E. coli	GCCACACUGGAACUGAGACACGGUCCAGACUCCUACGGGAGGCAGCAGUGG	
Probe 1660	3'-TGTACCTTCACTCTGTGCCGGTTGAGGATGCCCTCCGTCGTC-5'	
Probe 1659	3'-TGAGGATGCCCTCCGTCGTC-5'	
E. coli#s	504	535
CONS-90%	C CGGCUAACU GUGCCAGCAGCCCGCGUAUAC	
E. coli	CACCGCUAACUCCGUGCCAGCAGCCCGCGUAUACGG	
Probe 1661	3'-GCCGATTGAGGCACGGTCTGTGCGGCCATTAT-5'	
E. coli#s	764	810
CONS-90%	G CGAAGCGUGGGGAGC AACAGGAUUAUACCCUGGUAGUCCACGC	U
E. coli	GUGCGAAGCGUGGGGAGCAACAGGAUUAUACCCUGGUAGUCCACGC	CGU
Probe 1739	3'-GCTTTCGCACCCCTCGTTTCTAATCTATGGGGCCATCAGGTGCG-5'	

TABLE 1 (cont'd): 16S rRNA-TARGETED PROBES AND TARGET SEQUENCES

E. coli#s	1044	1114
CONS-90%		
E. coli	ACAGGUG UGCAUGG UGUGUCAGGUCGUG CGUGAG UGUUGGUUAAGUCCCGCAACGAGCGCAACCC	
Probe 1745	GAGACAGGUGGUGCAUGGUGGUGUCAGGUCGUGUGUUGAAGUUGGGUUAAGUCCCGCAACGAGCGCAACCCUUA	
Probe 1740	3'-TGTCCACCACCTACCAACAGCAGTCGA-5'	
Probe 1741	3'-TACGTACCGACAGCAGTCGAGCAGCGCACTTACAGCCCAATTTCAGGGCGTTGCTCGCGTTGGG-5'	
Probe 1742	3'-TACGTACCGACAGCAGTCGAGCAGCGCACTC-5'	
	3'-TACAGCCCAATTTCAGGGCGTTGCTCGCGTTGGG-5'	
E. coli#s	1188	1216
CONS-90%		
E. coli	GGA GACGUCAA UC UCAUG CCCUUA G	
Probe 1746	GGGAUGACGUCAAGUCAUGCAUGGCGCCUUAAGGACCA	
	3'-TACTGCAGTTTAGTAGTAGTACGGGGAATACT-5'	
E. coli#s	1369	1395
CONS-90%		
E. coli	CGGUGAAUACGUUC CGG CUUGUACACA	
Probe 1743	CCACGGUGAAUACGUUCGCGGCGCCUUGUACACA	
	3'-GCCACTTATGCAAGGGCGCCGGAACATG-5'	
E. coli#s	1492	1541
CONS-90%		
E. coli	AAGUCGUACAAGGUA CC UA GAA UG GG UGGAU ACCUCCUUU	
Probe 1637	GUGAAGUCGUACAAGGUACCGUAGGGGAACCUCCGUUGGAUACCUCCUUA-3'	
Probe 1639	3'-TTCAGCAATTGTTCCATTGGCA-5'	
Probe 1640	3'-TTCAGCAATTGTTCCATTGGCA-5'	
Probe 1641	3'-TTCAGCAATTGTTCCATTAGGCA-5'	
	3'-CCGACCTAGTGGAGGA-5'	

TABLE 2: 23S rRNA-TARGETED PROBES AND TARGET SEQUENCES

E. coli #s	442	481
CONS-90%	GACCGAUAG G AC AGUACCGUGAGGGAAAGG GAAAAG AC	
E.coli 23S	ACUGACCGAUAGUGAACCAGUACCGUGAGGGAAAGGCGAAAAGAAC	
Probe 1730	3'-CTGGCTATCRCTTGGTCATGGCRCTCCCTTTCCRCTTTTC-5'	
Probe 1731	3'-CTGGCTATCGCTTGGTCATGGCGCTCCCTTTCCGCTTTTC-5'	
E. coli #s	1049	1088
CONS-90%	A ACA C AGGA GUUGGCUUAGAAGCAGCCA C UU AAAGA G	
E.coli 23S	AGACAGCCAGGAUGUUGGCUUAGAAGCAGCCAUCAUUUAAAGAAAG	
Probe 1658	3'-GTTGGTCCTACAACCGAATCTTCGTCGGTGGGAAATTTCT-5'	
E. coli #s	1597	1639
CONS-90%	CGUACC AAACCGACACAGGU G G A A C AG	
E.coli 23S	UCAAAUCGUACCCCAAACCGACACAGGUGGUCAGGUAGAGAAUACCAAG	
Probe 1656	3'-CGGGCATGGCGTTTGGCTGTGTCCATC-5'	
Probe 1657	3'-GCATGGGGTTTGGCTGTGTCCACCAGTCCATCTCTTATGG-5'	
E. coli #s	1664	1703
CONS-90%	AAGGAACU GCAA U CCGUAACUUCGG A AAGG	
E.coli 23S	GUGAAGGAACUAGGC AAAAUGGUGCCGUAACUUCGGGAGAAGGCAC	
Probe 1653	3'-TTCCTTGATCCGTTTTACGGGGGCATTGAAGCCCTCTTCC-5'	
Probe 1654	3'-TTCCTTGAGCCGTTTTACTGGGGCATTGAAGCCCTCTTCC-5'	
Probe 1655	3'-TTCCTTGAGCCGTTTTACCACGGCATTGAAGCCCTCTTCC-5'	
E. coli #s	1831	1860
CONS-90%	GAC CCUGCCC GUGC GGAAGGUUAA G	
E.coli 23S	GACGCCUGCCCGGUGCCGGAAGGUUAAUUGAUGGGG	
Probe 1651	3'-CGGACGGGCCACGACCTTCCAATTCTCCTC-5'	
E. coli #s	1851	1890
CONS-90%	AGGUUAA G U G AAG A GAAGCC	
E.coli 23S	AGGUUAAUUGAUGGGGUUAGCGCAAGCGAAGCUCUUGAUCGAAGCC	
Probe 1652	3'-AATTAAC TACCCAGTCGCGTTTCGCTTCGGGGACTAGCTT-5'	

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TABLE 2 (cont'd): 23S rRNA-TARGETED PROBES  
AND TARGET SEQUENCES

E. coli #s	1889	1928
CONS-90%	GAAGCCCC GU AACGGCGGCGGUAACUAUAACGGUCCUAAGGU	
E.coli 23S	UCGAAGCCCCGGUAAACGGCGGCGGUAACUAUAACGGUCCUAAGGU	
Probe 1512	3'-TTCGGGGTCATTTGCCGCGGCATTGATATTGCCAGGATT-5'	
E. coli #s	1925	1968
CONS-90%	GUCCUAAGGUAGCGAAAUUCCUUGUCGGGUAAGUCCGACC GCACGAA	
E.coli 23S	GUCCUAAGGUAGCGAAAUUCCUUGUCGGGUAAGUCCGACCUGCACGAAU	
Probe 1256	3'-GATTCCATCGCTTTAAGGAACAGCCCATTCAGGCTGG-5'	
Probe 1511	3'-CCATCGCTTTAAGGAACAGCCCATTCAGGCTGGGCGTGC-5'	
Probe 1398	3'-GATTCCATCGCTTTAAGGAACAGCCAATTTATGGCTGG-5'	
E. coli #s	2442	2481
CONS-90%	AC C GGGGAUAACAGGCU AU C CC AG GU CA AUCG CG	
E.coli 23S	ACUCCGGGGGAUAACAGGCUAUACCGCCCAAGAGUUCUAUUCGACG	
Probe 1595	3'-GGCCCCATATTGTCCGACTATGGCGGGTTCTCAAGTATAGC-5'	
E. coli #s	2490	2529
CONS-90%	GUUUGGCACCUCGAUGUCGGCUC UC CAUCCUGGGGCGUG AG	
E.coli 23S	GGUGUUUGGCACCUCGAUGUCGGCUCAUCAUCCUGGGGCGUGAAG	
Probe 1600	3'-CAAACCGTGGAGCTACAGCCGAGTAGAGTAGGACCCCGAC-5'	
Probe 1601	3'-CAAACCGTGGAGCTACAGCCGAGTAGTGTAGGACCCCGAC-5'	
Probe 1602	3'-CAAACCGTGGAGCTACAGCCGAGTAGCGTAGGACCCCGAC-5'	
E. coli #s	2535	2574
CONS-90%	G GGUCCCAAGGGU GGCUGUUCGCC UUAAG GG ACC GA	
E.coli 23S	GUAGGUCCCAAGGGUAUGGCUGUUCGCCAUUUAAGUGGUACGCGA	
Probe 1598	3'-CCAGGGTTCCCATACCGACAAGCGGTAAATTTCCGCATGC-5'	
Probe 1599	3'-CCAGGGTTCCCAACCCGACAAGCGGGTAATTTCCCGTGC-5'	
E. coli #s	2577	2616
CONS-90%	G GAGCUGGGUU A AACGUCGUGAGACAGUU GGUC CUAUC	
E.coli 23S	GCGAGCUGGGUUAGAACGUCGUGAGACAGUUCGGUCCCUAUCUGC	
Probe 1596	3'-TCGACCCAAGTTTTGCAGCACTCTGTCAAGCCAGGGATAG-5'	
Probe 1597	3'-TCGACCCAAGTCTTGCACTCTGTCAAGCCAGGGATAG-5'	

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TABLE 3: DOT BLOT HYBRIDIZATION OF 16S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION										
			1738	1739	1659	1660	1661	1740	1741	1742	1743		
<i>Acinetobacter calcoaceticus</i>	GT0002	Purple	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Aeromonas sobria</i>	GT0007	gamma	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Alteromonas putrefaciens</i>	GT1945	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Citrobacter amalonaticus</i>	GT0690	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Citrobacter diversus</i>	GT0030	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Citrobacter freundii</i>	GT0687	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Edwardsiella tarda</i>	GT0569	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Enterobacter agglomerans</i>	GT0683	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Enterobacter cloacae</i>	GT0686	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Enterobacter sakazakii</i>	GT0062	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Escherichia coli</i>	GT1665	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Escherichia coli</i>	GT1592	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Escherichia coli</i>	GT1659	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Haemophilus influenza</i>	GT0244	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Haemophilus ducreyi</i>	GT0243	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Hafnia alvei</i>	GT0241	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Morgenella morganii</i>	GT0303	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Klebsiella pneumoniae</i>	GT1500	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Proteus mirabilis</i>	GT1496	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Providencia alcalifaciens</i>	GT0371	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Pseudomonas aeruginosa</i>	GT1909	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Salmonella arizona</i>	GT0799	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Salmonella typhimurium</i>	GT0389	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Serratia marcescens</i>	GT0392	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Shigella flexneri</i>	GT0798	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Vibrio parahaemolyticus</i>	GT0568	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Xanthomonas maltophilia</i>	GT0417	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Yersinia enterocolitica</i>	GT0419	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Alcaligenes faecalis</i>	GT0610	Purple	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Branhamella catarrhalis</i>	GT0014	beta	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Chromobacterium violaceum</i>	GT2022	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Kingella indologenes</i>	0246	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Moraxella osloensis</i>	GT0301	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Moraxella phenylpyruvica</i>	GT0302	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

TABLE 3 (cont'd): DOT BLOT HYBRIDIZATION OF 16S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION										
			1738	1739	1659	1660	1661	1740	1741	1742	1743		
Borrelia burgdorferi		Spiro	++++	++++	++++	++++	++++	++++	++++	++++	++++		
Borrelia turicatae		"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
Leptospira interrogans-pomona		"	++++	+	++++	++++	++++	++++	++++	++++	+		
Leptospira biflexa (Patoc-Patoc)		"	++++	+	++++	++++	++++	++++	++++	++++	+		
Leptospira biflexa (CDC)		"	++++	+	++++	++++	++++	++++	++++	++++	+		
Spirochaeta aurantia		"	++++	+	++++	++++	++++	++++	++++	++++	+		
Bacteroides fragilis	25285 Bact		++++	+	++++	++++	++++	++++	++++	++++	+		
Bacteroides fragilis	29771 "		++++	+	++++	++++	++++	++++	++++	++++	+		
Bacteroides thetaiotaomicron	0572 "		++++	+	++++	++++	++++	++++	++++	++++	+		
Bacteroides melanogonicus	0011 "		++++	+	++++	++++	++++	++++	++++	++++	+		
Flavobacterium meningosepticum	0237 "		++++	+	++++	++++	++++	++++	++++	++++	+		
Chlamydia psittaci		Chlam	-	++++	-	++++	++++	++++	++++	++++	++++		
Chlamydia trachomatis		"	-	++++	-	++++	++++	++++	++++	++++	++++		
Chlorobium limicola		Misc	++++	++++	++++	++++	++++	++++	++++	++++	++++		
Chloroflexus aurantiacus	Y400 "		++++	++++	++++	++++	++++	++++	++++	++++	++++		
Deinococcus radiodurans	2608 "		++++	++++	++++	++++	++++	++++	++++	++++	++++		
Planctomyces maris	2577 "		++++	++++	-	++++	++++	++++	++++	++++	-		
Normal Stool RNA			++++	++++	++++	++++	++++	++++	++++	++++	++++		
Mouse L-Cell			-	+	-	-	++	-	-	-	-		
Wheat Germ			-	-	-	-	++	-	-	-	-		
Normal Human Blood			-	-	-	-	++	-	-	-	-		
Candida lusitanae	403-87		-	-	-	-	++++	-	-	-	-		
Candida parapsilosis	882-88		-	-	-	-	++++	-	-	-	-		
Candida tropicalis	224-87		-	-	-	-	++++	-	-	-	-		
Candida albicans	1008-88		-	-	-	-	++++	-	-	-	-		
Candida albicans	223-87		-	-	-	-	++++	-	-	-	-		
Candida albicans	819-88		-	-	-	-	++++	-	-	-	-		

\* Inclusivity and Exclusivity data was determined after overnight exposures.

\*\* Each organism is represented by 100ng of CsTFA purified RNA.

\*\*\* Probe 1738 - hybridizations and washes were carried out at 37 C. +++ = positive control level of hybridization, + = barely detectable and - = zero, ND = not done.

TABLE 3 (cont'd): DOT BLOT HYBRIDIZATION OF 16S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION										
			1738	1739	1659	1660	1661	1740	1741	1742	1743		
Mycoplasma pneumoniae	ATCC15531	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-
Mycoplasma putrefaciens	ATCC15718	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	-	-
Peptostreptococcus productus	ATCC27340	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Planococcus citreus	ATCC14404	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Staphylococcus aureus	GT0399	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Staphylococcus aureus	GT1711	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Staphylococcus epidermidis	GT0401	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Staphylococcus haemolyticus	ATCC29970	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus agalactiae	GT0405	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus bovis	GT0668	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus faecalis	GT0406	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus morbillorum	GT2194	"	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus mutans	GT0412	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus pneumoniae	GT0408	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus salivarius	GT0410	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Streptococcus sanguis	GT0411	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Bifidobacterium dentium	GT0012	hig+C	-	+++	+++	+++	+++	+++	+++	+++	+++	ND	ND
Corynebacterium genitalium	GT0045	Gm +	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Corynebacterium glutamicum	GT2120	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Corynebacter. pseudodiphtheriticum	GT2119	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Corynebacterium pseudotuberculosis	GT2122	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Corynebacterium pyogenes	GT2121	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Corynebacterium xerosis	GT0046	"	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Mycobacterium bovis	BCC	"	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Mycobacterium kansasii		"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Nocardia asteroides		"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Rhodococcus rhodochrous	GT2191	Misc	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Aerococcus viridans	GT2116	Gm +	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Fusobacterium necrophorum	GT0238	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Fusobacterium praesnitzii	ATCC27768	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Gemella haemolysans	GT2118	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Hellobacillus mobilis		"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Phormidium ectocarpi		"	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Plectonema boryanum		Cyano	+++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++

TABLE 3 (cont'd): DOT BLOT HYBRIDIZATION OF 16S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION										
			1738	1739	1659	1660	1661	1740	1741	1742	1743		
<i>Neisseria gonorrhoeae</i>	GT0315	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Neisseria meningitidis</i>	GT0349	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Pseudomonas acidovorans</i>	GT0376	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Pseudomonas cepacia</i>	GT2015	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Rhodocyclus gelatinosa</i>	ATCC17013	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Vitreoscilla stercoraria</i>	GT0810	Purple	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Achromobacter xerosis</i>	ATCC33463	alpha	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Acidiphilium cryptum</i>	GT2021	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Agrobacterium tumefaciens</i>	ATCC23448	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Brucella abortus</i>	GT2025	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Flavobacterium capsulatum</i>	GT2023	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Mycoplana bullata</i>	GT2020	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Pseudomonas diminuta</i>	ATCC17023	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Rhodobacter sphaeroides</i>	ATCC35903	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Rhodospirillum rubrum</i>	ATCC25364	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Thiobacillus versutus</i>	ATCC 7757	delta	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Desulfovibrio desulfuricans</i>	GT2095	Gm -	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Cardiobacterium hominis</i>	GT2172	Gm -	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Francisella tularensis</i>	GT0022	Campy	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Campylobacter jejuni</i>	GT0026	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Campylobacter pylori</i>	GT0027	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Campylobacter sputorum</i>	GT0803	lowG+C	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Bacillus brevis</i>	GT0804	Gm +	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Bacillus subtilis</i>	ATCC25537	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Clostridium clostridioforme</i>	GT0567	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Clostridium sordellii</i>	ATCC 3587	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Clostridium sporogenes</i>	ATCC19401	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Clostridium histolyticum</i>	ATCC13124	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Clostridium perfringens</i>	ATCC25582	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Clostridium ramosum</i>	ATCC33403	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Kurthia zopfii</i>	GT0256	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Lactobacillus acidophilus</i>	GT0258	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Lactobacillus plantarum</i>	IG3299	"	++++	++++	++++	++++	++++	++++	++++	++++	++++		
<i>Listeria monocytogenes</i>			++++	++++	++++	++++	++++	++++	++++	++++	++++		

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TABLE 4: DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION									
			1730	1731	1658	1653	1654	1655	1651			
Acinetobacter calcoaceticus	GT0002	Purple	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Aeromonas sobria	GT0007	gamma	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Alteromonas putrefaciens	GT1945	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Citrobacter amalonaticus	GT0690	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Citrobacter diversus	GT0030	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Citrobacter freundii	GT0687	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Edwardsiella tarda	GT0569	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Enterobacter agglomerans	GT0683	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Enterobacter cloacae	GT0686	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Enterobacter sakazakii	GT0062	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Escherichia coli	GT1665	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Escherichia coli	GT1592	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Escherichia coli	GT1659	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Haemophilus influenza	GT0244	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Haemophilus influenza	ATCC33391	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Haemophilus ducreyi	GT0243	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Hafnia alvei	GT0241	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Morganella morganii	GT0303	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Klebsiella pneumoniae	GT1500	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Proteus mirabilis	GT1496	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Providencia alcalifaciens	GT0371	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Pseudomonas aeruginosa	GT1909	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Salmonella arizona	GT0799	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Salmonella typhimurium	GT0389	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Serratia marcescens	GT0392	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Shigella flexneri	GT0798	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Vibrio parahaemolyticus	GT0568	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Xanthomonas maltophilia	GT0417	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Yersinia enterocolitica	GT0419	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Alcaligenes faecalis	GT0610	Purple	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Branhamella catarrhalis	GT0014	beta	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Chromobacterium violaceum	GT2022	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Kingella indologenes	0246	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Moraxella osloensis	GT0301	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

TABLE 4 (cont'd): DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION									
			1730	1731	1658	1653	1654	1655	1651			
<i>Morexella phenylpyruvica</i>	GT0302	"	+++	+++	+	+++	+++	+++	+++	+++	+++	+++
<i>Neisseria gonorrhoeae</i>	GT0315	"	+++	+++	+++	-	+++	+++	+++	+		
<i>Neisseria meningitidis</i>	GT0349	"	+++	+++	+++	-	+++	+++	+++	+++	+++	+++
<i>Pseudomonas acidovorans</i>	GT0376	"	+++	+++	+++	-	+++	+++	+++	+++	+++	+++
<i>Pseudomonas cepacia</i>	GT2015	"	+++	+++	+++	-	-	++	-			
<i>Rhodocyclus gelatinosa</i>	ATCC17013	"	+++	+++	+++	-	++	-	++			
<i>Vitreoscilla stercoraria</i>		"	+++	+++	+++	-	+++	+++	+++	+++	+++	+++
<i>Achromobacter xerosis</i>	GT0810	Purple	+++	+++	+++	-	-	++	+++	+++	+++	+++
<i>Acidiphilium cryptum</i>	ATCC33463	alpha	+	-	++	-	-	-	++	+++	+++	+++
<i>Agrobacterium tumefaciens</i>	GT2021	"	+++	+++	+++	-	-	-	+++	+++	+++	+++
<i>Brucella abortus</i>	ATCC23448	"	+++	+++	+++	-	-	-	+++	+++	+++	+++
<i>Flavobacterium capsulatum</i>	GT2025	"	+++	+++	+++	-	-	-	+++	+++	+++	+++
<i>Mycoplana bullata</i>	GT2023	"	+++	+++	+	-	-	-	-	-	-	-
<i>Pseudomonas diminuta</i>	GT2020	"	+++	+++	+	-	-	-	-	-	-	-
<i>Rhodobacter sphaeroides</i>	ATCC17023	"	+++	+++	+++	-	-	-	+++	+++	+++	+++
<i>Rhodospirillum rubrum</i>	ATCC25903	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Thiobacillus versutus</i>	ATCC25364	"	+++	+++	+++	-	-	-	+++	+++	+++	+++
<i>Desulfovibrio desulfuricans</i>	ATCC 7757	delta	+++	+	++	+	++	-	+++	+++	+++	+++
<i>Cardiobacterium hominis</i>	GT2095	Gm -	+++	+++	-	-	+++	-	+++	+	+	+
<i>Francisella tularensis</i>	GT2172	Gm -	+++	+++	+++	-	-	-	+++	-	+	+
<i>Campylobacter jejuni</i>	GT0022	Campy	+++	+	+++	-	-	-	-	-	-	-
<i>Campylobacter pylori</i>	GT0026	"	+++	++	+++	-	-	-	+++	+++	+++	+++
<i>Campylobacter sputorum</i>	GT0027	"	+++	++	+++	-	-	-	-	-	-	-
<i>Bacillus brevis</i>	GT0803	lowG+C	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Bacillus subtilis</i>	GT0804	Gm +	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Clostridium clostridioforme</i>	ATCC25537	"	-	-	+	+++	+++	+++	+++	+++	+++	+++
<i>Clostridium sordellii</i>	GT0567	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Clostridium sporogenes</i>	ATCC 3587	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Clostridium histolyticum</i>	ATCC19401	"	+++	++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Clostridium perfringens</i>	ATCC13124	"	+++	+++	+++	-	+++	+++	+++	+++	+++	+++
<i>Clostridium ramosum</i>	ATCC25582	"	+++	+++	-	-	-	-	+++	+++	+++	+++
<i>Kurthia zopfii</i>	ATCC33403	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
<i>Lactobacillus acidophilus</i>	GT0256	"	+++	+++	-	+	+++	+++	+++	+++	+++	+++
<i>Lactobacillus plantarum</i>	GT0258	"	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

TABLE 4 (cont'd): DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION									
			1730	1731	1658	1653	1654	1655	1651			
<i>Listeria monocytogenes</i>	IG3299	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Mycoplasma pneumoniae</i>	ATCC15531	"	+++	++	+++	-	-	-	-	-		-
<i>Mycoplasma putrefaciens</i>	ATCC15718	"	+++	++	++	-	-	-	-	-		-
<i>Peptostreptococcus productus</i>	ATCC27340	"	-	-	++	+++	+++	+++	+++	+++		+++
<i>Planococcus citreus</i>	ATCC14404	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Staphylococcus aureus</i>	GT0399	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Staphylococcus aureus</i>	GT1711	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Staphylococcus epidermidis</i>	GT0401	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Staphylococcus haemolyticus</i>	ATCC29970	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Streptococcus agalactiae</i>	GT0405	"	+++	+++	-	+++	+++	+++	+++	+++		+++
<i>Streptococcus bovis</i>	GT0668	"	+++	+++	-	+++	+++	+++	+++	+++		+++
<i>Streptococcus faecalis</i>	GT0406	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Streptococcus morbillorum</i>	GT2194	"	+++	++	+++	+++	+++	+++	+++	+++		+++
<i>Streptococcus mutans</i>	GT0412	"	+++	++	-	+++	+++	+++	+++	+++		+++
<i>Streptococcus pneumoniae</i>	GT0408	"	+++	+++	-	+++	+++	+++	+++	+++		+++
<i>Streptococcus salivarius</i>	GT0410	"	+++	++	-	+++	+++	+++	+++	+++		+++
<i>Streptococcus sanguis</i>	GT0411	"	+++	+++	-	+++	+++	+++	+++	+++		+++
<i>Bifidobacterium dentium</i>	GT0012	hiG+C	+++	+++	-	-	-	-	-	-		+++
<i>Corynebacterium genitalium</i>	GT0045	Gm +	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Crynebacterium glutamicum</i>	GT2120	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Corynebacterium pseudodiphtheriticum</i>	GT2119	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Corynebacterium pseudotuberculosis</i>	GT2122	"	+++	+++	-	++	+++	+++	+++	+++		+++
<i>Corynebacterium pyogenes</i>	GT2121	"	+	-	+	+++	+++	+++	+++	+++		+++
<i>Corynebacterium xerosis</i>	GT0046	"	-	-	+	+++	+++	+++	+++	+++		+++
<i>Mycobacterium bovis</i>	BCC	"	-	-	+	+++	+++	+++	+++	+++		+++
<i>Mycobacterium kansasii</i>	GT2191	"	+	-	+	+++	+++	+++	+++	+++		+++
<i>Nocardia asteroides</i>	GT2191	"	+++	+++	+++	-	-	-	-	-		+++
<i>Rhodococcus rhodochrous</i>	GT2116	Misc	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Aerococcus viridans</i>	GT0238	Gm +	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Fusobacterium necrophorum</i>	ATCC27768	"	+++	-	++	++	++	++	++	++		+++
<i>Fusobacterium prausnitzii</i>	GT2118	"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Gemella haemolysans</i>		"	+++	+++	+++	+++	+++	+++	+++	+++		+++
<i>Helicobacillus mobilis</i>		"	+++	++	+++	+++	+++	+++	+++	+++		+++
<i>Phormidium ectocarpi</i>		Cyano	+	++	-	+++	+++	+++	+++	+++		+++

TABLE 4 (cont'd): DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION							
			1730	1731	1658	1653	1654	1655	1651	
Plectonema boryanum		"	++	++	-	++	+++	++	-	
Borrelia burgdorferi		Spiro	+++	++	++	-	-	-	+++	
Borrelia turicatae		"	+++	+++	+++	-	-	-	+++	
Leptospira interrogans-pomona		"	+++	++	+++	-	-	-	++	
Leptospira biflexa (Patoc-Patoc)		"	+++	++	+++	-	-	-	+++	
Leptospira biflexa (CDC)		"	+++	++	+++	-	-	-	+++	
Spirochaeta aurantia		"	+++	++	+++	-	-	-	+++	
Bacteroides fragilis	25285 Bact	"	++	++	+	-	-	-	+++	
Bacteroides fragilis	29771	"	++	++	+	-	-	-	+++	
Bacteroides thetaiotaomicron	0572	"	+	+	+	-	-	-	+++	
Bacteroides melaninogenicus	0011	"	-	-	+	++	++	-	+++	
Flavobacterium meningosepticum	0237	"	+	-	+	-	-	-	+++	
Chlamydia psittaci	LGV	Chlam	-	-	-	-	-	-	-	
Chlamydia trachomatis		"	+++	+++	-	-	-	-	-	
Chlorobium limicola		Misc.	+++	+++	+	+++	+++	+++	+++	
Chloroflexus aurantiacus	Y400	"	+	-	++	-	++	-	+	
Deinococcus radiodurans	2608	"	+++	+++	+++	-	-	-	+	
Planctomyces maris	2577	"	-	-	+++	-	-	-	-	
Normal Stool RNA			+++	++	+++	++	+++	++	+++	
Mouse L-Cell			-	-	-	-	-	-	-	
Wheat Germ			+	++	-	-	-	-	-	
Normal Human Blood			-	-	-	-	-	-	-	
Candida lusitanae	403-87		-	-	-	-	-	-	-	
Candida parapsilosis	882-88		-	-	-	-	-	-	-	
Candida tropicalis	224-87		-	-	-	-	-	-	-	
Candida albicans	1008-88		-	-	-	-	-	-	-	
Candida albicans	223-87		-	-	-	-	-	-	-	
Candida albicans	819-88		-	-	-	-	-	-	-	

\* Inclusivity and Exclusivity data was determined after overnight exposures.

\*\* Each organism is represented by 100ng of CsTFA purified RNA. +++ = positive level of hybridization, + = barely detectable and - = zero, ND = not done.

TABLE 4 (cont'd): DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION									
			1512	1256	1398	1600	1601	1602	1596	1597		
<i>Acinetobacter calcoaceticus</i>	GT0002	Purple	+++	+++	+	+++	+++	+++	+++	++		
<i>Aeromonas sobria</i>	GT0007	gamma	+++	+++	++	+++	+++	+++	+++	++		
<i>Alteromonas putrefaciens</i>	GT1945	"	+++	+++	++	+++	+++	+++	+++	++		
<i>Citrobacter amalonaticus</i>	GT0690	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Citrobacter diversus</i>	GT0030	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Citrobacter freundii</i>	GT0687	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Edwardsiella tarda</i>	GT0569	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Enterobacter agglomerans</i>	GT0683	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Enterobacter cloacae</i>	GT0686	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Enterobacter sakazakii</i>	GT0062	"	+++	+++	++	+++	+++	+++	+++	++		
<i>Escherichia coli</i>	GT1665	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Escherichia coli</i>	GT1592	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Haemophilus influenza</i>	GT1659	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Haemophilus influenza</i>	GT0244	"	++	+++	+	+++	+++	+++	+++	++		
<i>Haemophilus ducreyi</i>	ATCC33391	"	ND	ND	ND	+++	+++	+++	+++	++		
<i>Hafnia alvei</i>	GT0243	"	+++	+++	++	+++	+++	+++	+++	++		
<i>Morganella morganii</i>	GT0241	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Klebsiella pneumoniae</i>	GT0303	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Proteus mirabilis</i>	GT1500	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Providencia alcalifaciens</i>	GT1496	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Pseudomonas aeruginosa</i>	GT0371	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Salmonella arizona</i>	GT1909	"	+++	+++	++	+++	+++	+++	+++	++		
<i>Salmonella typhimurium</i>	GT0799	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Serratia marcescens</i>	GT0389	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Shigella flexneri</i>	GT0392	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Vibrio parahaemolyticus</i>	GT0798	"	+++	+++	++	+++	+++	+++	+++	++		
<i>Xanthomonas maltophilia</i>	GT0568	"	+++	+++	++	+++	+++	+++	+++	++		
<i>Yersinia enterocolitica</i>	GT0417	"	+++	+++	++	+++	+++	+++	+++	++		
<i>Alcaligenes faecalis</i>	GT0419	Purple	+++	+++	++	+++	+++	+++	+++	++		
<i>Branhamella catarrhalis</i>	GT0610	beta	+++	+++	+	+++	+++	+++	+++	++		
<i>Chromobacterium violaceum</i>	GT0014	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Kingella indologenes</i>	GT2022	"	+++	+++	+	+++	+++	+++	+++	++		
<i>Moraxella osloensis</i>	0246	"	+++	+++	+	+++	+++	+++	+++	++		
	GT0301	"	+++	+++	+	+++	+++	+++	+++	++		

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TABLE 4 (cont'd): DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION										
			1512	1256	1398	1600	1601	1602	1596	1597			
<i>Morexella phenylpyruvica</i>	GT0302	"	+++	+++	+	+++	++	+++	+++	+++			
<i>Neisseria gonorrhoeae</i>	GT0315	"	+++	+++	+	+++	++	+++	+++	++			
<i>Neisseria meningitidis</i>	GT0349	"	+++	+++	+	+++	+++	+++	+++	++			
<i>Pseudomonas acidovorans</i>	GT0376	"	+++	+++	+	+++	+++	+++	+++	++			
<i>Pseudomonas cepacia</i>	GT2015	"	+++	+++	+	+++	+++	+++	+++	++			
<i>Rhodocyclus gelatinosa</i>	ATCC17013	"	+++	+++	+	+++	+++	+++	+++	++			
<i>Vitreoscilla stercoraria</i>		"	+++	++	+	+++	+++	+++	+++	+++			
<i>Achromobacter xerosis</i>	GT0810	Purple	+++	++	+	+++	+++	+++	+++	+++			
<i>Acidiphilium cryptum</i>	ATCC33463	alpha	+++	++	+	+++	+++	+++	+++	+++			
<i>Agrobacterium tumefaciens</i>	GT2021	"	+++	++	+	++	++	+++	++	+++			
<i>Brucella abortus</i>	ATCC23448	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Flavobacterium capsulatum</i>	GT2025	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Mycoplasma bullata</i>	GT2023	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Pseudomonas diminuta</i>	GT2020	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Rhodobacter sphaeroides</i>	ATCC17023	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Rhodospirillum rubrum</i>	ATCC25903	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Thiobacillus versutus</i>	ATCC25364	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Desulfovibrio desulfuricans</i>	ATCC 7757	delta	+++	+++	+	+++	+++	+++	+++	+			
<i>Cardiobacterium hominis</i>	GT2095	Gm -	+++	+++	+	+++	+++	+++	+++	++			
<i>Francisella tularensis</i>	GT2172	Gm -	+++	+++	+	+++	+++	+++	+++	+++			
<i>Campylobacter jejuni</i>	GT0022	Campy	+++	-	+++	+++	++	+++	+++	+++			
<i>Campylobacter pylori</i>	GT0026	"	+++	-	+++	+++	+++	+++	+++	+++			
<i>Campylobacter sputorum</i>	GT0027	"	+++	-	+++	+++	+++	+++	+++	+++			
<i>Bacillus brevis</i>	GT0803	lowG+C	+++	+++	+	+++	+++	+++	+++	+++			
<i>Bacillus subtilis</i>	GT0804	Gm +	+++	+++	+	+++	+++	+++	+++	+++			
<i>Clostridium clostridioforme</i>	ATCC25537	"	+++	+++	+	+++	++	+++	+++	+++			
<i>Clostridium sordellii</i>	GT0567	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Clostridium sporogenes</i>	ATCC 3587	"	+++	+++	+	+++	++	+++	+++	+++			
<i>Clostridium histolyticum</i>	ATCC19401	"	+++	+++	+	+	+	+++	+++	+++			
<i>Clostridium perfringens</i>	ATCC13124	"	+++	-	+	+++	+++	+++	+++	+++			
<i>Clostridium ramosum</i>	ATCC25582	"	+++	-	+	+++	++	+++	+++	+++			
<i>Kurthia zopfii</i>	ATCC33403	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Lactobacillus acidophilus</i>	GT0256	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Lactobacillus plantarum</i>	GT0258	"	+++	+++	+	+++	+++	+++	+++	+++			

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TABLE 4 (cont'd): DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	Strain	div	PROBE HYBRIDIZATION										
			1512	1256	1398	1600	1601	1602	1596	1597			
<i>Listeria monocytogenes</i>	IG3299	"	+++	+++	+	+++	+++	+++	++	+++			
<i>Mycoplasma pneumoniae</i>	ATCC15531	"	+++	-	+	++	-	+++	-	-			
<i>Mycoplasma putrefaciens</i>	ATCC15718	"	+++	+	-	+++	++	+++	+++	+++			
<i>Peptostreptococcus productus</i>	ATCC27340	"	+++	+++	-	++	++	+++	+++	+++			
<i>Planococcus citreus</i>	ATCC14404	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Staphylococcus aureus</i>	GT0399	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Staphylococcus aureus</i>	GT1711	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Staphylococcus epidermidis</i>	GT0401	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Staphylococcus haemolyticus</i>	ATCC29970	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Streptococcus agalactiae</i>	GT0405	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Streptococcus bovis</i>	GT0668	"	+++	++	+	+++	+++	+++	+++	+++			
<i>Streptococcus faecalis</i>	GT0406	"	+++	++	+	+++	+++	+++	+++	+++			
<i>Streptococcus morbillorum</i>	GT2194	"	+++	+++	+	++	+++	+++	++	+++			
<i>Streptococcus mutans</i>	GT0412	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Streptococcus pneumoniae</i>	GT0408	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Streptococcus salivarius</i>	GT0410	"	+++	+++	+	+++	+++	+++	+++	++			
<i>Streptococcus sanguis</i>	GT0411	"	ND	ND	+	+++	+++	+++	+++	+++			
<i>Bifidobacterium dentium</i>	GT0012 h1G+C	"	-	+++	+	+++	+++	+++	+++	+++			
<i>Corynebacterium genitalium</i>	GT0045	Gm +	++	+++	+	+++	+++	+++	+++	+++			
<i>Corynebacterium glutamicum</i>	GT2120	"	++	+++	+	+++	+++	+++	+++	+++			
<i>Corynebacterium pseudodiphtheriticum</i>	GT2119	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Corynebacterium pseudotuberculosis</i>	GT2122	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Corynebacterium pyogenes</i>	GT2121	"	++	++	+	+++	++	+++	+++	+++			
<i>Corynebacterium xerosis</i>	GT0046	"	+	++	+	+++	++	+++	++	+++			
<i>Mycobacterium bovis</i>	BCG	"	+	++	+	+++	+	+++	++	+++			
<i>Mycobacterium kansasii</i>	GT2191	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Nocardia asteroides</i>		"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Rhodococcus rhodochrous</i>		"	++	+++	+	+++	++	+++	+++	+++			
<i>Aerococcus viridans</i>	GT2116	Misc	+++	+++	+	+++	+++	+++	+++	+++			
<i>Fusobacterium necrophorum</i>	GT0238	Gm +	++	+++	+	+++	++	+++	+++	+++			
<i>Fusobacterium prausnitzii</i>	ATCC27768	"	+++	+++	+	+++	+++	+++	+++	+++			
<i>Gemella haemolysans</i>	GT2118	"	+++	+	+	+++	++	+++	+++	+++			
<i>Helicobacillus mobilis</i>		"	+++	+++	+	+	+	+++	++	+++			
<i>Phormidium ectocarpi</i>		Cyano	+++	+++	+	+	+	+++	++	+++			

TABLE 4 (cont'd): DOT BLOT HYBRIDIZATION OF 23S rRNA-TARGETED PROBES

Genus species	strain	div	PROBE HYBRIDIZATION									
			1512	1256	1398	1600	1601	1602	1596	1597		
Plectonema boryanum		"	++++	+++	+	+	+	++++	++	++		
Borrelia burgdorferi		Spiro	++++	+++	+	+++	+++	++++	+++	++		
Borrelia turicatae		"	++++	+++	+	+++	+++	++++	+++	++		
Leptospira interrogans-pomona		"	++++	+++	+	+++	+++	++++	+++	++		
Leptospira biflexa (Patoc-Patoc)		"	++++	+++	+	+++	+++	++++	+++	++		
Leptospira biflexa (CDC)		"	++++	+++	+	+++	+++	++++	+++	++		
Spirochaeta aurantia		"	++++	+++	+	+++	+++	++++	+++	++		
Bacteroides fragilis	25285 Bact		++++	+++	+	+++	+++	++++	+++	++		
Bacteroides fragilis	29771		++++	+++	+	+++	+++	++++	+++	++		
Bacteroides thetaiotaomicron	0572	"	++++	+++	+	+	+	++++	+++	++		
Bacteroides melaninogenicus	0011	"	++++	+++	+	+	+	++++	+++	++		
Flavobacterium meningosepticum	0237	"	++++	+++	+	+++	+++	++++	+++	++		
Chlamydia psittaci		Chlam	-	+++	-	+++	+++	+++	+++	++		
Chlamydia trachomatis	LGV	"	-	+++	-	+++	+++	+++	+++	++		
Chlorobium limicola		Misc.	+++	+++	+	+++	+++	+++	+++	++		
Chloroflexus aurantiacus	Y400	"	+++	+++	+	+++	+++	+++	+++	++		
Deinococcus radiodurans	2608	"	+++	+++	+	+++	+++	+++	+++	++		
Planctomyces maris	2577	"	+++	+++	+	+++	+++	+++	+++	++		
Normal Stool RNA			+++	+++	-	+++	+++	+++	+++	++		
Mouse L-Cell			-	-	-	-	-	-	-	-		
Wheat Germ			-	-	-	-	-	-	-	-		
Normal Human Blood			-	-	-	-	-	-	-	-		
Candida lusitanae	403-87		+	-	-	-	-	-	-	-		
Candida parapsilosis	882-88		+	-	-	-	-	-	-	-		
Candida tropicalis	224-87		+	-	-	-	-	-	-	-		
Candida albicans	1008-88		+	-	-	-	-	-	-	-		
Candida albicans	223-87		+	-	-	-	-	-	-	-		
Candida albicans	819-88		+	-	-	-	-	-	-	-		

\* Inclusivity and Exclusivity data was determined after overnight exposures.

\*\* Each organism is represented by 100ng of CsTFA purified RNA. +++ = positive level of hybridization, + = barely detectable and - = zero, ND - not done.

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TABLE 5: DOT BLOT HYBRIDIZATION - GRAM POSITIVE &amp; GRAM NEGATIVE PROBES

Genus species	strain	div	PROBE HYBRIDIZATION									
			16S RNA-TARGET					23S RNA-TARGET				
			1744	1745	1746	1657	1656	1598	1599	1595	1595	1595
<i>Acinetobacter calcoaceticus</i>	GT0002	Purple	-	-	-	++++	-	++	-	++++	++++	++++
<i>Aeromonas sobria</i>	GT0007	gamma	-	-	-	++++	-	++	-	++++	++++	++++
<i>Alteromonas putrefaciens</i>	GT1945	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Citrobacter amalonaticus</i>	GT0690	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Citrobacter diversus</i>	GT0030	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Citrobacter freundii</i>	GT0687	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Edwardsiella tarda</i>	GT0569	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Enterobacter agglomerans</i>	GT0683	"	-	-	-	++++	-	++	-	++++	++++	++++
<i>Enterobacter cloacae</i>	GT0686	"	+	-	-	++++	-	++	+	++++	++++	++++
<i>Enterobacter sakazakii</i>	GT0062	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Escherichia coli</i>	GT1665	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Escherichia coli</i>	GT1592	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Escherichia coli</i>	GT1659	"	-	-	-	++++	+	++	+	++++	++++	++++
<i>Haemophilus influenza</i>	ATCC33391	"	-	-	-	++++	++++	+	+	++++	++++	++++
<i>Haemophilus ducreyi</i>	GT0243	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Hafnia alvei</i>	GT0241	"	-	-	-	++++	-	++++	+	++++	++++	++++
<i>Morganella morganii</i>	GT0303	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Klebsiella pneumoniae</i>	GT1500	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Proteus mirabilis</i>	GT1496	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Providencia alcalifaciens</i>	GT0371	"	-	-	-	++++	-	++	+	++++	++++	++++
<i>Pseudomonas aeruginosa</i>	GT1909	"	-	-	-	++++	-	++	+	++++	++++	++++
<i>Salmonella arizona</i>	GT0799	"	-	-	-	++++	-	++	+	++++	++++	++++
<i>Salmonella typhimurium</i>	GT0389	"	-	-	-	++++	-	++	+	++++	++++	++++
<i>Serratia marcescens</i>	GT0392	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Shigella flexneri</i>	GT0798	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Vibrio parahaemolyticus</i>	GT0568	"	-	-	-	++++	-	++++	+	++++	++++	++++
<i>Xanthomonas maltophilia</i>	GT0417	"	-	-	-	+	-	++++	+	++++	++++	++++
<i>Yersinia enterocolitica</i>	GT0419	"	-	-	-	++++	-	++++	+	++++	++++	++++
<i>Alcaligenes faecalis</i>	GT0610	Purple	-	-	-	-	++++	+	+	++++	++++	++++
<i>Branhamella catarrhalis</i>	GT0014	beta	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Chromobacterium violaceum</i>	GT2022	"	-	-	-	++++	+	++++	+	++++	++++	++++
<i>Kingella indologenes</i>	0246	"	-	-	-	++++	-	++++	-	++++	++++	++++
<i>Moraxella osloensis</i>	GT0301	"	-	-	-	++++	-	++++	+	++++	++++	++++
<i>Morexella phenylpyruvica</i>	GT0302	"	-	-	-	++++	-	++++	-	++++	++++	++++

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TABLE 5: (cont'd) DOT BLOT HYBRIDIZATION - GRAM POSITIVE &amp; GRAM NEGATIVE PROBES

Genus species	strain	div	PROBE HYBRIDIZATION						
			16S RNA-TARGET			23S RNA-TARGET			
			1744	1745	1746	1657	1656	1598	1595
<i>Borrelia burgdorferi</i>		Spiro	-	+	+++	-	++++	++	-
<i>Borrelia turicatae</i>		"	-	++	++++	-	++++	++++	+
<i>Leptospira interrogans-pomona</i>		"	-	+	-	-	++++	++++	++
<i>Leptospira biflexa (Patoc-Patoc)</i>		"	-	-	-	-	++++	++++	++
<i>Leptospira biflexa (CDC)</i>		"	-	-	-	-	++++	++++	+
<i>Spirochaeta aurantia</i>		"	++++	-	-	-	++++	++	+
<i>Bacteroides fragilis</i>	25285 Bact		++++	-	-	-	-	++++	+
<i>Bacteroides fragilis</i>	29771 "		++++	-	-	-	-	++++	+
<i>Bacteroides thetaiotaomicron</i>	0572 "		+	-	-	-	-	++++	+
<i>Bacteroides melaninogenicus</i>	0011 "		++++	-	-	-	-	++++	+
<i>Flavobacterium meningosepticum</i>	0237 "		++++	-	-	-	+	++++	-
<i>Chlamydia psittaci</i>	Chlamy		-	-	-	-	-	+	++
<i>Chlamydia trachomatis</i>	LGV		-	-	-	-	-	++	++
<i>Chlorobium limicola</i>	Misc		++++	-	-	-	+++	-	-
<i>Chloroflexus aurantiacus</i>	Y400		++++	-	-	-	+++	-	+++
<i>Deinococcus radiodurans</i>	2608 "		+++	-	-	-	+++	-	+++
<i>Planctomyces maris</i>	2577 "		-	-	-	-	-	-	-
Normal Stool RNA			+++	+++	++	++	+++	+	++
Mouse L-Cell			-	-	-	-	-	-	-
Wheat Germ			-	-	-	-	-	-	-
Normal Human Blood			-	-	-	-	-	-	-
<i>Candida lusitanae</i>	403-87		-	-	-	-	-	-	-
<i>Candida parapsilosis</i>	882-88		-	-	-	-	-	-	-
<i>Candida tropicalis</i>	224-87		-	-	-	-	-	-	-
<i>Candida albicans</i>	1008-88		-	-	-	-	-	-	-
<i>Candida albicans</i>	223-87		-	-	-	-	-	-	-
<i>Candida albicans</i>	819-88		-	-	-	-	-	-	-

\* Inclusivity and Exclusivity data was determined after overnight exposures.

\*\* Each organism is represented by 100ng of CsTFA purified RNA.

\*\*\* +++ = positive level of hybridization, + = barely detectable and - = zero. Probe 1746 was hybridized at 37 C and washes at 50 C.

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TABLE 5: (cont'd) DOT BLOT HYBRIDIZATION - GRAM POSITIVE &amp; GRAM NEGATIVE PROBES

Genus species	strain	div	PROBE HYBRIDIZATION						
			16S RNA-TARGET		23S RNA-TARGET				
			1744	1745	1746	1657	1656	1598	1599 1595
Mycoplasma pneumoniae	ATCC15531	"	-	++++	++++	-	-	-	+++
Mycoplasma putrefaciens	ATCC15718	"	-	++++	+++	-	-	-	+++
Peptostreptococcus productus	ATCC27340	"	++++	++++	++++	-	+	-	+++
Planococcus citreus	ATCC14404	"	++++	++++	++++	-	++++	+	+++
Staphylococcus aureus	GT0399	"	++++	++++	++++	-	++++	+	+++
Staphylococcus aureus	GT1711	"	++++	++++	++++	-	++++	+	+++
Staphylococcus epidermidis	GT0401	"	++++	++++	++++	-	++++	+	+++
Staphylococcus haemolyticus	ATCC29970	"	++++	++++	++++	-	++++	+	+++
Streptococcus agalactiae	GT0405	"	++++	++++	++++	-	++++	-	+++
Streptococcus bovis	GT0668	"	++++	++++	++++	-	++++	-	+++
Streptococcus faecalis	GT0406	"	++++	++++	++++	-	++++	-	+++
Streptococcus morbillorum	GT2194	"	++++	++++	++++	-	++++	+	+++
Streptococcus mutans	GT0412	"	++++	++++	++++	-	++++	-	+++
Streptococcus pneumoniae	GT0408	"	++++	++++	++++	-	++++	-	+++
Streptococcus salivarius	GT0410	"	++++	++++	++++	-	++++	-	+++
Streptococcus sanguis	GT0411	"	++++	++++	++++	-	++++	-	+++
Bifidobacterium dentium	GT0012	hiG+C	-	++++	+++	++++	-	-	+++
Corynebacterium genitalium	GT0045	Gm +	++++	++++	++++	++++	+	-	+++
Corynebacterium glutamicum	GT2120	"	++++	++++	++++	++++	-	-	+++
Corynebacterium pseudodiphtheriticum	GT2119	"	++++	++++	++++	-	+++	+	+++
Corynebacterium pseudotuberculosis	GT2122	"	++++	++++	++++	-	+++	-	+++
Corynebacterium pyogenes	GT2121	"	++++	++++	++++	-	-	+	+++
Corynebacterium xerosis	GT0046	"	++++	++++	++	++++	-	-	+++
Corynebacterium bovis	BCG	"	++++	++++	-	++++	-	-	+++
Mycobacterium kansasii	GT2191	"	++++	++++	++	++++	-	-	+++
Nocardia asteroides	GT2191	"	++++	++++	-	++++	-	+++	+++
Rhodococcus rhodochrous	GT2116	Misc	++++	++++	+	++++	-	-	+++
Aerococcus viridans	GT0238	Gm +	++++	++++	++++	-	++	++	+++
Fusobacterium necrophorum	ATCC27768	"	++++	++++	++	++	-	+	+++
Fusobacterium prausnitzii	GT2118	"	++++	++++	++++	-	+++	+++	+++
Gemella haemolysans		"	++++	++++	++++	-	+++	+++	+++
Helicobacter mobilis		"	++++	++++	++++	-	+++	+++	+++
Phormidium ectocarpi		Cyano	++++	++++	++	-	-	-	+++
Plectonema boryanum		"	++++	++++	-	-	+	-	+++

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TABLE 5: (cont'd) DOT BLOT HYBRIDIZATION - GRAM POSITIVE &amp; GRAM NEGATIVE PROBES

Genus species	strain	div	PROBE HYBRIDIZATION					
			16S RNA-TARGET		23S RNA-TARGET			
			1744	1745	1746	1657	1656	1598 1599 1595
<i>Neisseria gonorrhoeae</i>	GT0315	"	-	-	-	++++	++++	++++
<i>Neisseria meningitidis</i>	GT0349	"	-	-	-	++++	++++	++++
<i>Pseudomonas acidovorans</i>	GT0376	"	-	-	-	++++	++++	++++
<i>Pseudomonas cepacia</i>	GT2015	"	-	-	-	++	++++	++++
<i>Rhodocyclus gelatinosa</i>	ATCC17013	"	-	-	-	-	-	++++
<i>Vitreoscilla stercoraria</i>	GT0810	Purple	-	-	-	++++	++++	++++
<i>Achromobacter xerosis</i>	ATCC33463	alpha	-	-	-	++	-	-
<i>Acidiphilium cryptum</i>	GT2021	"	++	-	-	++++	++++	++
<i>Agrobacterium tumefaciens</i>	ATCC23448	"	++	-	-	++++	++++	++++
<i>Brucella abortus</i>	GT2025	"	+	-	-	++++	++	++
<i>Flavobacterium capsulatum</i>	GT2023	"	-	-	-	++++	++	-
<i>Mycoplana bullata</i>	GT2020	"	-	-	-	++++	++	+
<i>Pseudomonas diminuta</i>	GT2020	"	-	-	-	++++	++	++
<i>Rhodobacter sphaeroides</i>	ATCC17023	"	-	-	-	++++	++	++
<i>Rhodospirillum rubrum</i>	ATCC25903	"	++++	-	-	++++	+	++++
<i>Thiobacillus versutus</i>	ATCC25364	"	++	-	-	++++	++	++
<i>Desulfovibrio desulfuricans</i>	ATCC 7757	delta	-	-	-	-	++	++
<i>Cardiobacterium hominis</i>	GT2095	Misc	-	-	-	++++	++	+
<i>Francisella tularensis</i>	GT2172	Gm -	-	-	-	++++	++	++++
<i>Campylobacter jejuni</i>	GT0022	Campy	-	-	-	-	++++	++
<i>Campylobacter pylori</i>	GT0026	"	-	-	-	-	++++	++
<i>Campylobacter sputorum</i>	GT0027	"	-	-	-	-	++	++
<i>Bacillus brevis</i>	GT0803	lowG+C	++++	++++	++++	++++	+	++++
<i>Bacillus subtilis</i>	GT0804	Gm +	++++	++++	++++	++++	+	++++
<i>Clostridium clostridioforme</i>	ATCC25537	"	++++	++++	++++	-	++++	-
<i>Clostridium sordellii</i>	GT0567	"	++++	++++	++++	-	++++	-
<i>Clostridium sporogenes</i>	ATCC 3587	"	++++	++++	++++	-	++++	-
<i>Clostridium histolyticum</i>	ATCC19401	"	++++	++++	++++	-	++++	-
<i>Clostridium perfringens</i>	ATCC13124	"	++++	++++	++++	-	++++	-
<i>Clostridium ramosum</i>	ATCC25582	"	++++	++++	++++	-	++++	-
<i>Kurthia zopfii</i>	ATCC33403	"	++++	++++	++++	-	++++	+
<i>Lactobacillus acidophilus</i>	GT0256	"	++++	++++	++++	-	++++	-
<i>Lactobacillus plantarum</i>	GT0258	"	++++	++++	++++	-	++++	-
<i>Listeria monocytogenes</i>	IG3299	"	++++	++++	++++	-	++++	-

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What is claimed is:

1. A nucleic acid fragment capable of hybridizing to rRNA or rDNA of eubacteria.
- 5 2. The nucleic acid fragment of claim 1 wherein said fragment is not capable of hybridizing to rRNA or rDNA of Mouse L cells, wheat germ, human blood or Candida albicans.
- 10 3. The nucleic acid fragment of claim 2, wherein said fragment is complementary to at least 90% of a sequence comprising any ten consecutive nucleotides within probes selected from the group consisting of 1638, 1642, 1643, 1738, 1744, 1659, 1660, 1661, 1739, 1740, 1741, 1742, 1745, 1746, 1743, 1637, 1639, 1640, 1641, 15 1730, 1731, 1658, 1656, 1657, 1653, 1654, 1655, 1651, 1652, 1512, 1256, 1398, 1511, 1595, 1600, 1601, 1602, 1598, 1599, 1596, 1597.
- 20 4. The nucleic acid fragment of claim 2, wherein said fragment is homologous to at least 90% of a sequence comprising any ten consecutive nucleotides within probes selected from the group consisting of 1638, 1642, 1643, 1738, 1744, 1659, 1660, 1661, 1739, 1740, 1741, 1742, 1745, 1746, 1743, 1637, 1639, 1640, 1641, 1730, 1731, 1658, 1656, 1657, 1653, 1654, 1655, 1651, 1652, 1512, 1256, 1398, 1511, 1595, 1600, 1601, 1602, 1598, 1599, 1596, 1597.
- 25 5. A set of probes comprising at least two nucleic acids fragments, at least one of which is selected from the group consisting of 1638, 1642, 1643, 1738, 1744, 1659, 1660, 1661, 1739, 1740, 1741, 1742, 1745, 1746, 1743, 1637, 1639, 1640, 1641, 1730, 1731, 1658, 1656, 1657, 1653, 1654, 1655, 1651, 1652, 1512, 1256, 1398, 1511, 1595, 1600, 1601, 1602, 1598, 1599, 1596, 1597 and their 30 complementary sequences.

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- 5
6. The nucleic acid fragment of claim 1 which is probe 1638 or its complementary sequence.
7. The nucleic acid fragment of claim 1 which is probe 1642 or its complementary sequence.
8. The nucleic acid fragment of claim 1 which is probe 1643 or its complementary sequence.
- 10
9. The nucleic acid fragment of claim 1 which is probe 1738 or its complementary sequence.
10. The nucleic acid fragment of claim 1 which is probe 1744 or its complementary sequence.
- 15
11. The nucleic acid fragment of claim 1 which is probe 1659 or its complementary sequence.
12. The nucleic acid fragment of claim 1 which is probe 1660 or its complementary sequence.
- 20
13. The nucleic acid fragment of claim 1 which is probe 1661 or its complementary sequence.
- 25
14. The nucleic acid fragment of claim 1 which is probe 1739 or its complementary sequence.
15. The nucleic acid fragment of claim 1 which is probe 1740 or its complementary sequence.
- 30
16. The nucleic acid fragment of claim 1 which is probe 1741 or its complementary sequence.
- 35
17. The nucleic acid fragment of claim 1 which is probe 1742 or its complementary sequence.

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18. The nucleic acid fragment of claim 1 which is probe 1745 or its complementary sequence.
- 5 19. The nucleic acid fragment of claim 1 which is probe 1746 or its complementary sequence.
20. The nucleic acid fragment of claim 1 which is probe 1743 or its complementary sequence.
- 10 21. The nucleic acid fragment of claim 1 which is probe 1637 or its complementary sequence.
- 15 22. The nucleic acid fragment of claim 1 which is probe 1639 or its complementary sequence.
23. The nucleic acid fragment of claim 1 which is probe 1640 or its complementary sequence.
- 20 24. The nucleic acid fragment of claim 1 which is probe 1641 or its complementary sequence.
- 25 25. The nucleic acid fragment of claim 1 which is probe 1730 or its complementary sequence.
26. The nucleic acid fragment of claim 1 which is probe 1731 or its complementary sequence.
- 30 27. The nucleic acid fragment of claim 1 which is probe 1658 or its complementary sequence.
28. The nucleic acid fragment of claim 1 which is probe 1656 or its complementary sequence.

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29. The nucleic acid fragment of claim 1 which is probe 1657 or its complementary sequence.
- 5 30. The nucleic acid fragment of claim 1 which is probe 1653 or its complementary sequence.
31. The nucleic acid fragment of claim 1 which is probe 1654 or its complementary sequence.
- 10 32. The nucleic acid fragment of claim 1 which is probe 1655 or its complementary sequence.
33. The nucleic acid fragment of claim 1 which is probe 1651 or its complementary sequence.
- 15 34. The nucleic acid fragment of claim 1 which is probe 1652 or its complementary sequence.
- 20 35. The nucleic acid fragment of claim 1 which is probe 1512 or its complementary sequence.
36. The nucleic acid fragment of claim 1 which is probe 1256 or its complementary sequence.
- 25 37. The nucleic acid fragment of claim 1 which is probe 1398 or its complementary sequence.
38. The nucleic acid fragment of claim 1 which is probe 1511 or its complementary sequence.
- 30 39. The nucleic acid fragment of claim 1 which is probe 1595 or its complementary sequence.
- 35 40. The nucleic acid fragment of claim 1 which is probe 1600 or its complementary sequence.

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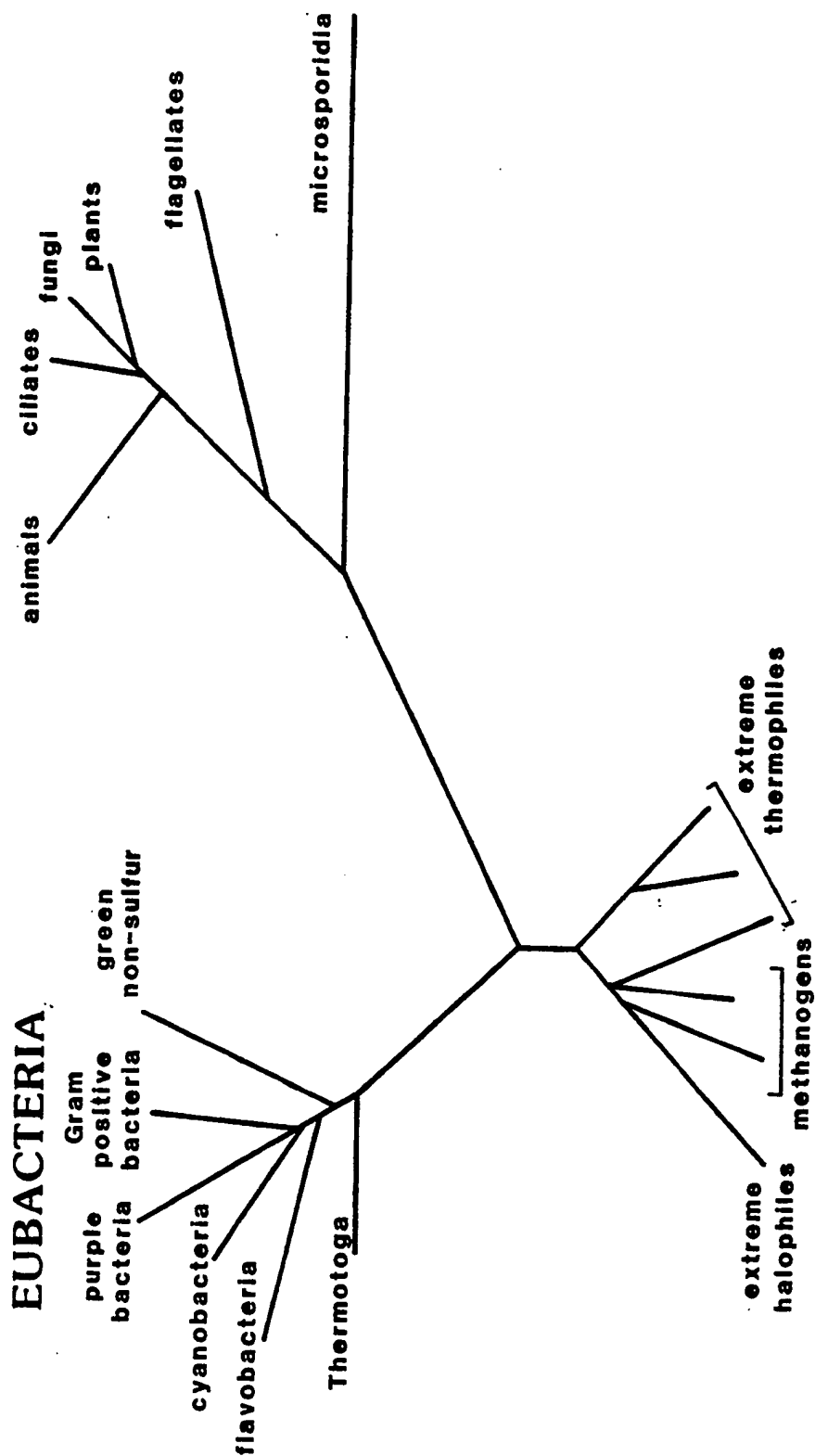
41. The nucleic acid fragment of claim 1 which is probe 1601 or its complementary sequence.
- 5 42. The nucleic acid fragment of claim 1 which is probe 1602 or its complementary sequence.
43. The nucleic acid fragment of claim 1 which is probe 1598 or its complementary sequence.
- 10 44. The nucleic acid fragment of claim 1 which is probe 1599 or its complementary sequence.
45. The nucleic acid fragment of claim 1 which is probe 1596 or its complementary sequence.
- 15 46. The nucleic acid fragment of claim 1 which is probe 1597 or its complementary sequence.
- 20 47. A method of detecting the presence of eubacteria in a sample comprising:
- 25 a) contacting said sample with at least one nucleic acid fragment under conditions that allow said fragment to hybridize to rRNA or rDNA of said eubacteria, if present in said sample, to form hybrid nucleic acid complexes not to rRNA or rDNA of non-eubacteria; and
- 30 b) detecting said hybrid nucleic acid complexes as an indication of the presence of said eubacteria in said sample.
- 35 48. The method of claim 47 wherein said nucleic acid fragment of said contacting step is selected from the group of probes consisting of 1638, 1642, 1643, 1738, 1744, 1659, 1660, 1661, 1739, 1740, 1741, 1742, 1745, 1746, 1743, 1637, 1639, 1640, 1641, 1730, 1731, 1658, 1656, 1657, 1653, 1654, 1655, 1651, 1652, 1512, 1256, 1398, 1511, 1595, 1600, 1601, 1602, 1598, 1599, 1596, 1597.

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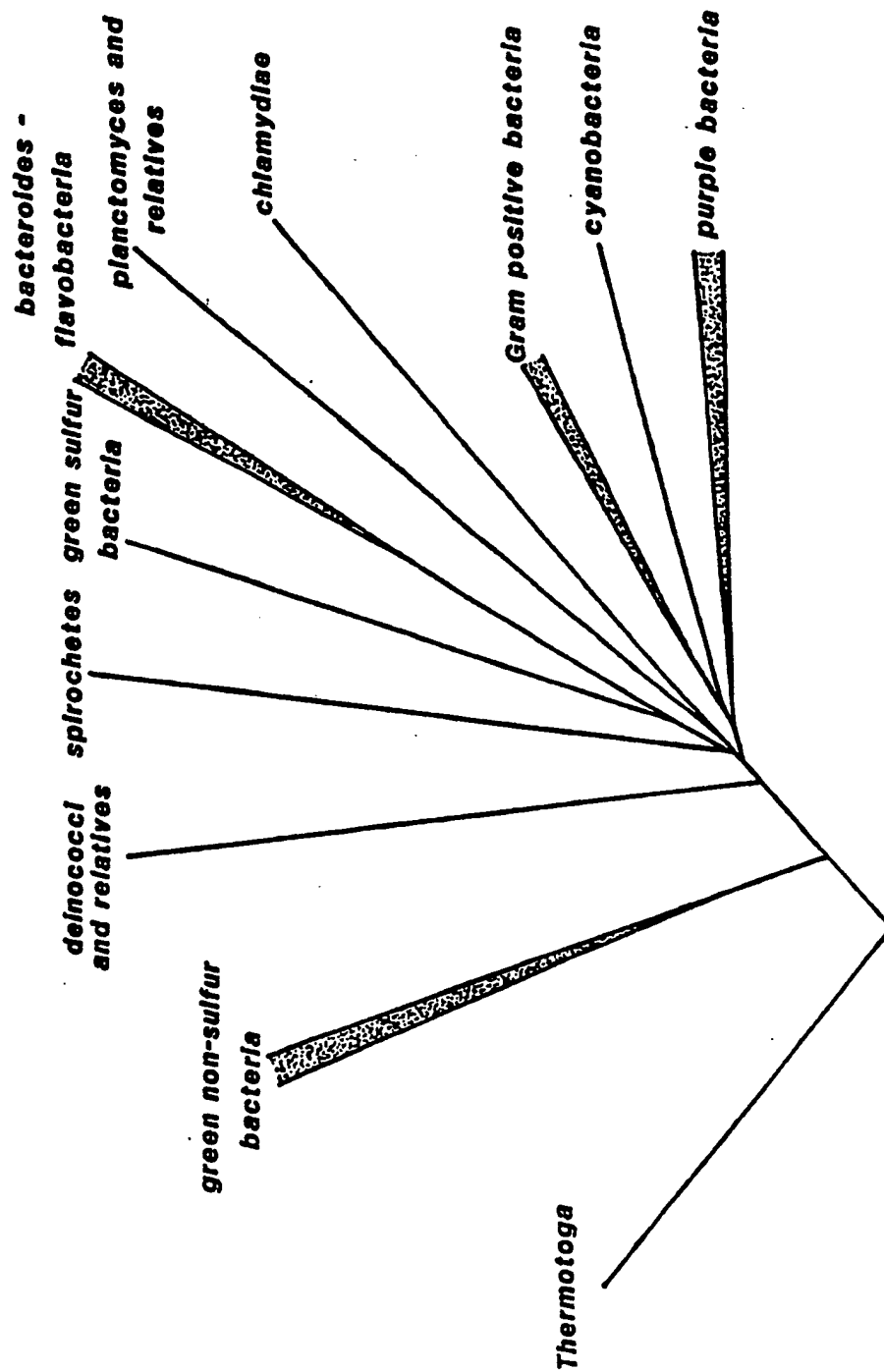
49. The method of claim 47 wherein said eubacteria are gram-positive and said nucleic acid fragment is selected from the group of probes consisting of 1599, 1656, 1744, 1745 and 1746.
- 5
50. The method of claim 47 wherein said eubacteria are gram-negative and said nucleic acid fragment is selected from the group of probes consisting of 1599, 1656, 1744, 1745 and 1746.
- 10
51. The method of claim 47 wherein said contacting step involves a nucleic acid fragment selected from the group consisting of probe 1638, probe 1642 and probe 1643 and said detecting step involves further contacting said sample with a second nucleic acid fragment selected from the group of probes consisting of 1637, 1639, 1640 and 1641 and amplifying eubacterial 16S rRNA or 16S rRNA gene sequences by the polymerase chain reaction.
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**FIGURE 1: THE THREE KINGDOMS**  
**EUKARYOTES**




**SUBSTITUTE SHEET**

**FIGURE 2: THE EUBACTERIAL KINGDOM**

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US 90/03004

<b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) <sup>6</sup> According to International Patent Classification (IPC) or to both National Classification and IPC <b>IPC5: C 12 Q 1/68</b>		
<b>II. FIELDS SEARCHED</b>		
Minimum Documentation Searched <sup>7</sup>		
Classification System	Classification Symbols	
IPC5	C 12 Q; C 07 H	
Documentation Searched other than Minimum Documentation to the extent that such Documents are Included in Fields Searched <sup>8</sup>		
<b>III. DOCUMENTS CONSIDERED TO BE RELEVANT<sup>9</sup></b>		
Category *	Citation of Document, <sup>11</sup> with Indication, where appropriate, of the relevant passages <sup>12</sup>	Relevant to Claim No. <sup>13</sup>
X  Y	EP, A1, 0245129 (INSTITUT PASTEUR) 11 November 1987, see especially the abstract  --	1-5,21, 47,48, 51, 3-5,21, 51
X  Y	EP, A1, 0250662 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 7 January 1988, see the whole document  --	1,2, 47  3-5,21, 48,51
A	EP, A1, 0277237 (TORAY INDUSTRIES, INC.) 10 August 1988, see the whole document  --	1-51
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p><sup>*</sup> Special categories of cited documents: <sup>10</sup></p> <p><sup>"A"</sup> document defining the general state of the art which is not considered to be of particular relevance</p> <p><sup>"E"</sup> earlier document but published on or after the international filing date</p> <p><sup>"L"</sup> document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p><sup>"O"</sup> document referring to an oral disclosure, use, exhibition or other means</p> <p><sup>"P"</sup> document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p><sup>"T"</sup> later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p><sup>"X"</sup> document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p><sup>"Y"</sup> document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p><sup>"&amp;"</sup> document member of the same patent family</p> </div> </div>		
<b>IV. CERTIFICATION</b>		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
28th September 1990		17. 10. 90
International Searching Authority		Signature of Authorized Officer
EUROPEAN PATENT OFFICE		 H. Ballesteros

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III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	SCIENCE, Vol. 243, March 1989, Edward F. DeLong et al.: "Phylogenetic Stains: Ribosomal RNA-Based Probes for the Identification of Single Cells", see page 1360	1,2, 47
Y		3-5,21, 48,51
	--	
X	Dialog Informational Service, file 154: Medline 83-90, accession no. 06930668, Medline accession no. 89232668, Chen K. et al: "Broad range DNA probes for detecting and amplifying eubacterial nucleic acids", & FEMS Microbiol Lett Jan 1 1989, 48(1) p19-24	1,2
Y		3-5,21, 48,51
	--	
X	Dialog Information Service, file 154: Medline 83-90, accession no. 06470172, Medline accession no. 88115172, Giovannoni S.J. et al: "Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells", & J Bacteriol (UNITED STATES) Feb 1988, 170 (2) p720-6	1
Y		3-5,21, 48,51
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**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO. PCT/US 90/03004**

SA 38253

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The members are as contained in the European Patent Office EDP file on 28/08/90  
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A1- 0245129	11/11/87	AU-D- 7232587	20/10/87
		FR-A-B- 2596774	09/10/87
		JP-T- 1500001	12/01/89
		WO-A- 87/05907	08/10/87
EP-A1- 0250662	07/01/88	NONE	
EP-A1- 0277237	10/08/88	WO-A- 88/00618	28/01/88

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